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DISTRIBUTION OF SOME COMMON DOMESTIC RAT ECTOPARASITES IN THE UNITED STATES

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The classic studies on murine typhus, which Maxcy and his co-workers began during the period 1925 to 1929 (Maxcy 1929), stimulated increasingly better reporting of this disease. The number of reported cases of murine typhus rose rapidly from 510 in 1930 to 2,787 in 1941, and to a high of 5,401 in 1944 (Fig. 1). It is probable that a much greater number of cases actually occurred.

In the period 1931 to 1944, great advances were made concerning the epidemiology and control of murine typhus. In the early 1930's, Dyer, Badger, and their associates at the National Institutes of Health demonstrated the importance of rat ectoparasites, particularly of the oriental rat flea, in the transmission of murine typhus from rat to rat, and from rat to man.

In 1944, DDT was tested as a rat ectoparasite insecticide in San Antonio, Tex. Davis (1945, 1947) demonstrated that populations of the oriental rat flea, *Xenopsylla cheopis* (Rothschild), could be controlled with this new insecticide, and that the incidence of murine typhus dropped remarkably in areas where this flea was controlled. Similar experiments were carried out in 1945 by workers of the Communicable Disease Center in Savannah, Ga. (Ludwig and Nicholson, 1947). In 1945, plague was controlled in the port district of Casablanca, Morocco, by the combined use of DDT residual sprays and 10 percent DDT dust to control rat fleas (Anonymous, 1945). As a result of these demonstrations, late in 1945 and in 1946 a cooperative murine typhus control program was initiated by the health departments of each of the southeastern states and the Communicable Disease Center of the Public Health Service. The success of this control program (which included rat poisoning, rat trapping, and ratproofing, but consisted primarily of DDT dusting) may be measured by the remarkable decrease in the number of cases of typhus in the 8 years since 1944 (Fig. 1).

ECTOPARASITE COLLECTIONS

A program of ectoparasite collection and determination was established as part of the over-all evaluation of this murine typhus control program. The largest number of collections of ectoparasites was made in the southeastern states where the greatest number of murine typhus cases occurred (Fig. 2). The distributional data are, therefore, much more complete for the southeastern states, where in-

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tensive surveys have been conducted for a number of years, than for the other parts of the country. The determination of the majority of the ectoparasites was made in the various states, but sizeable numbers of specimens were also determined in the Atlanta, Ga., laboratories of the Communicable Disease Center. Records of the determinations of rat ectoparasites from all over the United States, Puerto Rico, and Hawaii were sent to Atlanta for coding on machine record cards and for statistical studies. This paper presents data on the distribution of ectoparasites collected during this nation-wide program, with additional information from the literature.

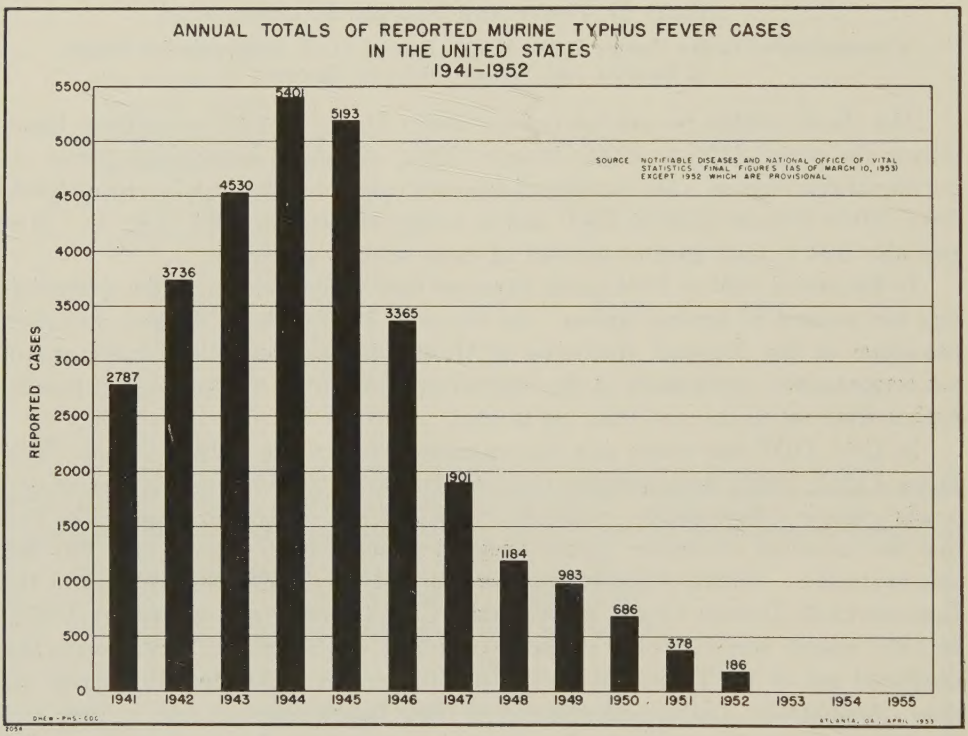

FIG. 1. Annual Totals of Reported Murine Typhus Fever Cases in the United States, 1941 to 1952.

RAT FLEAS

Four species of fleas have been found commonly on domestic rats, *Rattus rattus* (Linn.) and *Rattus norvegicus* (Erxleben) : the oriental rat flea, *X. cheopis;* the northern rat flea, *Nosopsyllus fasciatus* (Bosc) ; the mouse flea, *Leptopsylla segnis* (Schönherr) ; and the sticktight flea, *Echidnophaga gallinacea* (Westwood). The first three of these species are Old World fleas which probably were introduced into the New World on domestic rats. *E. gallinacea* may be a native flea, or it may have been introduced on rats or poultry.

The oriental rat flea (*X. cheopis*) (Fig. 3) probably is the most important species involved in the transmission of both plague and murine typhus. It was originally described from Egypt and the Sudan in 1903, and soon afterwards was reported from the San Francisco Bay area of California during the outbreaks of

plague in the early 1900's (see Mitzmain, 1907; Doane, 1908, and Fox, 1908).* This flea was found in port cities in Texas, Louisiana, and Florida by Public Health Service officials carrying out plague eradication in these states in the early 1920's (see Surgeon General, 1921 and 1922). Later, in the 1920's and 1930's, Public Health Service officials made surveys along the entire coast of the United States and demonstrated that *X. cheopis* occurs in all the major port cities of this country such as Seattle, Tacoma, Los Angeles, Boston, Providence, New York, Philadelphia, Norfolk, Savannah, and Jacksonville (see Fricks, 1936; Hundley and Nasi, 1944; Trimble and Sherrard, 1935; Fox and Sullivan, 1925; Robinson, 1913; Williams, 1929; Vogel and Cadwallader, 1935; Fox, 1931; and Rumreich and Wynn, 1945).

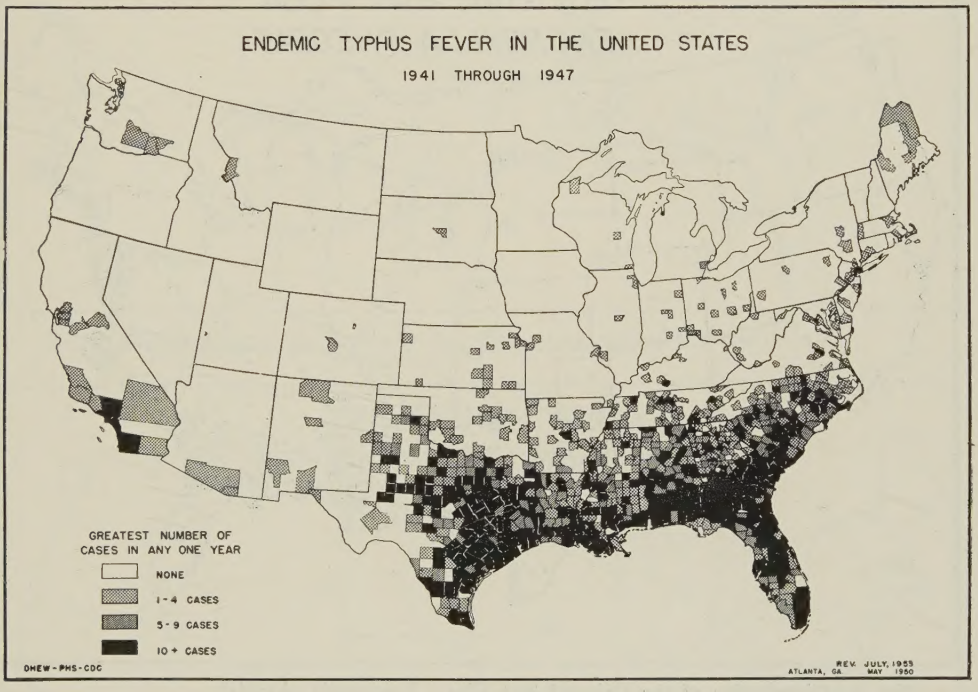

FIG. 2. Distribution of Endemic Typhus Fever in the United States, 1941 through 1947. These represent reported cases. Those north of Virginia, Tennessee, Kentucky, Texas and southern California are either other diseases, generally of rickettsial nature, misidentified as murine typhus fever or were acquired farther south. So, also, are those reported from Arizona and New Mexico.

In the 1930's and 1940's, a number of entomologists and parasitologists found *X. cheopis* in university towns in the Midwest, including St. Paul, Minn. (Owen, 1936); Ames, Iowa (Roudabush and Becker, 1934); Urbana, Ill., Youngstown, Ohio (Ewing and Fox, 1938); and Indianapolis, Ind. (Cable, 1943). In the early 1940's, field teams of the Plague Investigations Laboratory in San Francisco added many western records including Denver, Salt Lake City, Albuquerque, Phoenix, and many localities in California (Prince, 1943). Since 1945, the very

*Each dot on the distribution maps represents a single county in which one or more specimens of the particular ectoparasite have been collected.

extensive murine typhus control programs of the various state health departments and the Communicable Disease Center have added numerous county records in all the southeastern states and more recently in a number of western and northern states such as Utah, Wyoming, Colorado, Ohio, and New Hampshire.

The northern rat flea (*N. fasciatus*) (Fig. 4) was originally described from the Old World. In contrast to the oriental rat flea, the northern rat flea was first recorded in North America from Mammoth Cave, Ky. (Taschenberg, 1880) and Ames, Iowa (Osborn, 1896), both records being previous to 1900, followed by records from Mountain View, Calif., Washington, D. C., and Ottawa, Canada in 1904 (Baker, 1904) then by numerous records from San Francisco, 1907 to 1909

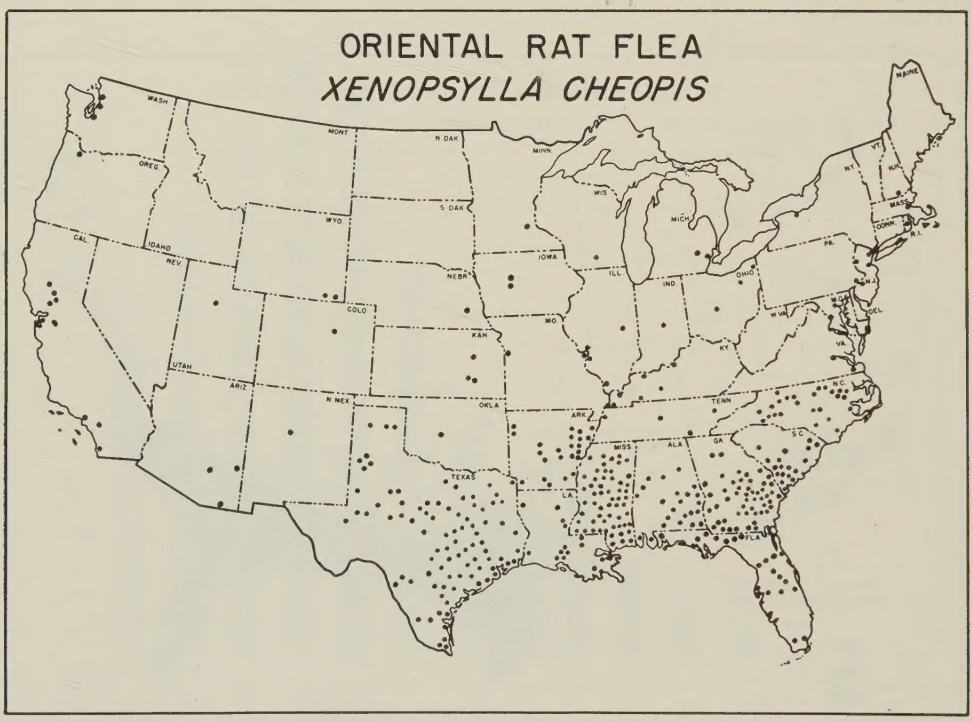

FIG. 3. Distribution of the Oriental Rat Flea (*Xenopsylla cheopis*).

(Doane, 1908; Fox, 1909), and subsequently from several port cities and numerous inland localities. As the name indicates, the northern rat flea is more abundant in the northern half of the United States and becomes progressively less common in southern United States although occasional specimens are found in almost every locality where collections are made for any length of time except in southern Florida and extreme southern Texas. In Rhode Island, Knutson and Szymkowicz (1952) found that 85 percent of all fleas collected on rats were the northern rat flea.

The sticktight flea (*E. gallinacea*) (Fig. 5) was originally described from Ceylon. It is a bird flea found very abundantly on domestic fowl and also is found in large numbers on mammalian hosts. As a result, it is found commonly on domestic rats in southern United States from coast to coast (Fig. 5) but becomes progressively rarer north of a line drawn along the northern boundaries of Mary-

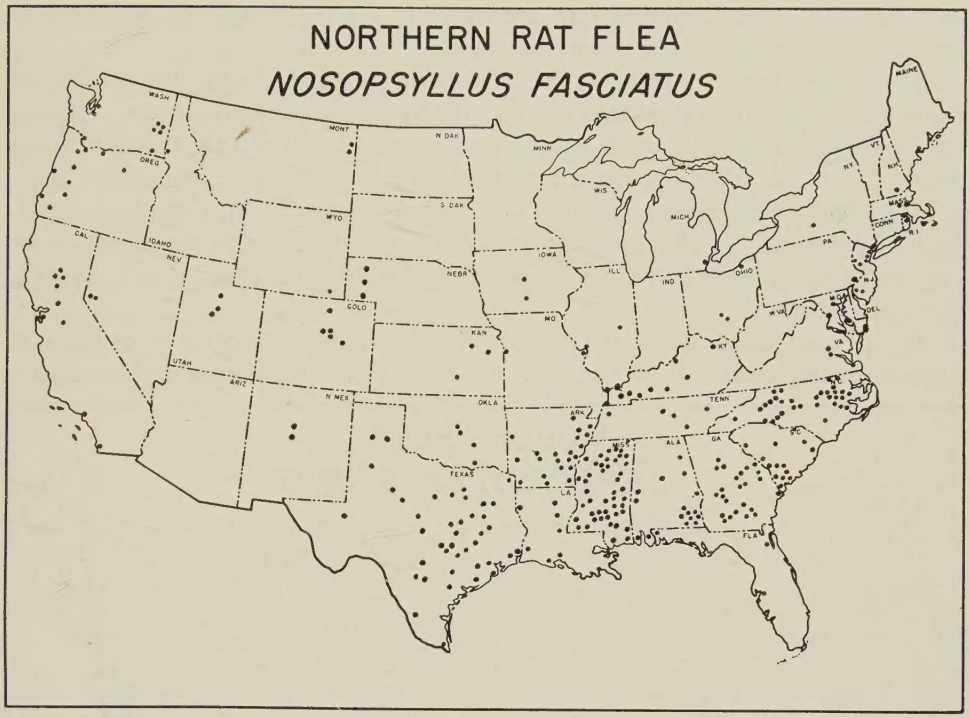

FIG. 4. Distribution of the Northern Rat Flea (*Nosopsyllus fasciatus*).

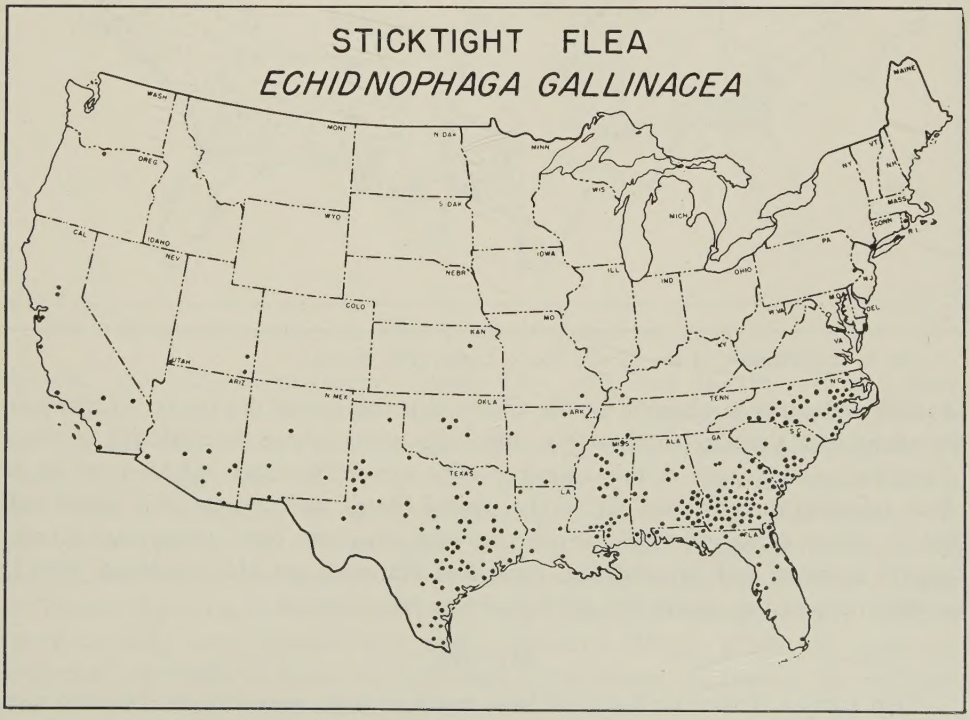

FIG. 5. Distribution of the Sticktight Flea (*Echidnophaga gallinacea*).

land, Missouri, and California. In southwestern United States, *E. gallinacea* is found on an amazing number of wild rodents.

The mouse flea (*L. segnis*) (Fig. 6), which was originally described from Europe, has been introduced into this country by means of rats on boats. On the Pacific coast, it is still abundant only in port towns such as Seattle and San Francisco. On the distribution maps, the other dots in western United States, such as Marshfield, Oreg.; Smith River, Fresno, and Williams, Calif.; Greenlee County, Ariz.; Lordsburg, N. M.; and Denver, Colo., are due to single specimens or to very small collections of this species made on domestic rats, house mice, shrews, and wild rodents.

In eastern United States, the mouse flea also has been reported from seacoast cities for many years. However, with the more extensive murine typhus control

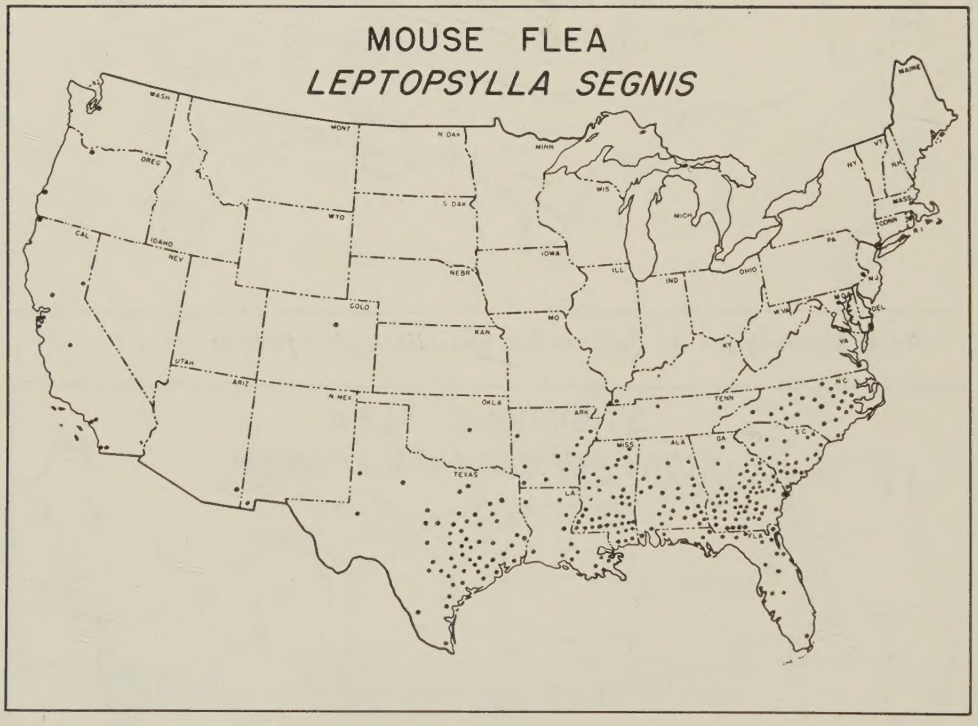

FIG. 6. Distribution of the Mouse Flea (*Leptopsylla segnis*).

projects and surveys inland it is now known that the mouse flea occurs in many of the counties of the nine southeastern states, the states where the majority of cases of murine typhus occur in this country. The mouse flea does not seem to be at all abundant in the northern part of the United States, and perhaps it is significant that in rather extensive recent surveys in such states as New Jersey and Rhode Island (Knutson and Szymkowicz, 1952, and Hansens and Hadjinicolaou, 1952), no specimens of the mouse flea were found in recent years.

RAT LICE

Two species of sucking lice have been taken in large numbers on domestic rats in the United States: the spiny rat louse, *Polyplax spinulosa* (Burmeister), and the tropical rat louse, *Hoplopleura oenomydis* Ferris.

It is probable that the spiny rat louse (*P. spinulosa*) (Fig. 7) occurs wherever domestic rats are found in the United States. This species has been found on domestic rats throughout the world and in every state in the United States where sizeable collections of rat ectoparasites have been made. On the map, the greater number of dots in such states as Georgia, Mississippi, and Texas reflect both intensive surveys and the larger number of counties in these areas.

The tropical rat louse (*H. oenomydis*) (Fig. 8), on the other hand, appears to be a more recent introduction in the United States as indicated by its occurrence in the larger seaport cities such as Seattle, San Francisco, Galveston, New Orleans, Mobile, Miami, Jacksonville, Savannah, New Bern, and Norfolk. Its spread inland has been along the main arteries of commerce, into such urban areas as

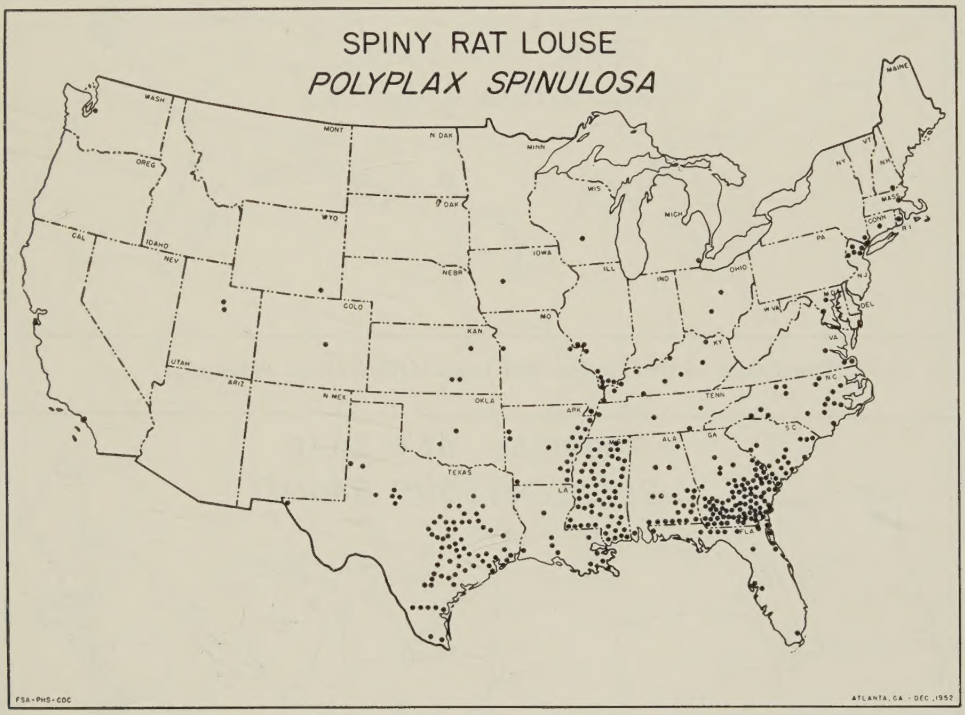

FIG. 7. Distribution of the Spiny Rat Louse (*Polyplax spinulosa*).

Atlanta, Nashville, Montgomery, Kansas City, and Fort Worth, where it may have been carried on domestic rats from port cities by truck or railroads. Its maximum spread inland known to the present writers is Ames, Iowa (Pritchard, 1947); Kansas City, Mo.; Nashville, Tenn.; and Richmond, Va. The last three records are based on specimens in the Communicable Disease Center collections. *H. oenomydis* is usually found in the large cities, but it is rare or absent in many rural areas in the South. For example, it has not been found in Brooks, Decatur, Grady, or Thomas Counties in Georgia although well-trained entomologists have watched for it carefully over a period of 3 years (Morlan, 1952). While the spiny rat louse may eventually be found in every state in the Union, the present data indicate that the tropical rat louse, which was originally described from specimens collected in Africa and the Philippine Islands, may be restricted to the southern part of the United States except for occasional specimens or infestations.

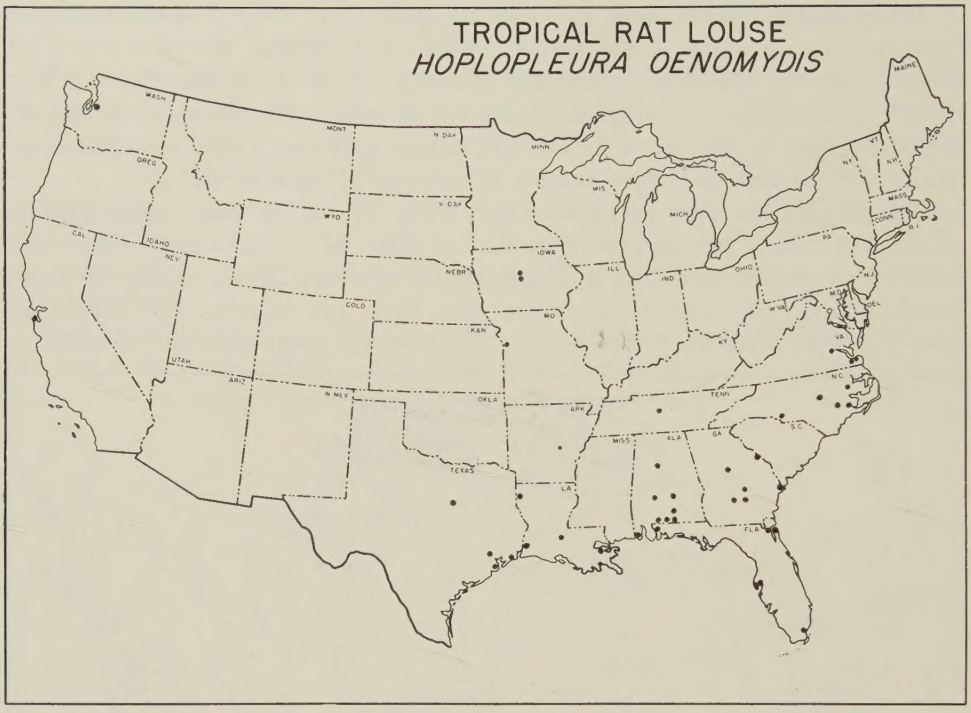

FIG. 8. Distribution of the Tropical Rat Louse (*Hoplopleura oenomydis*).

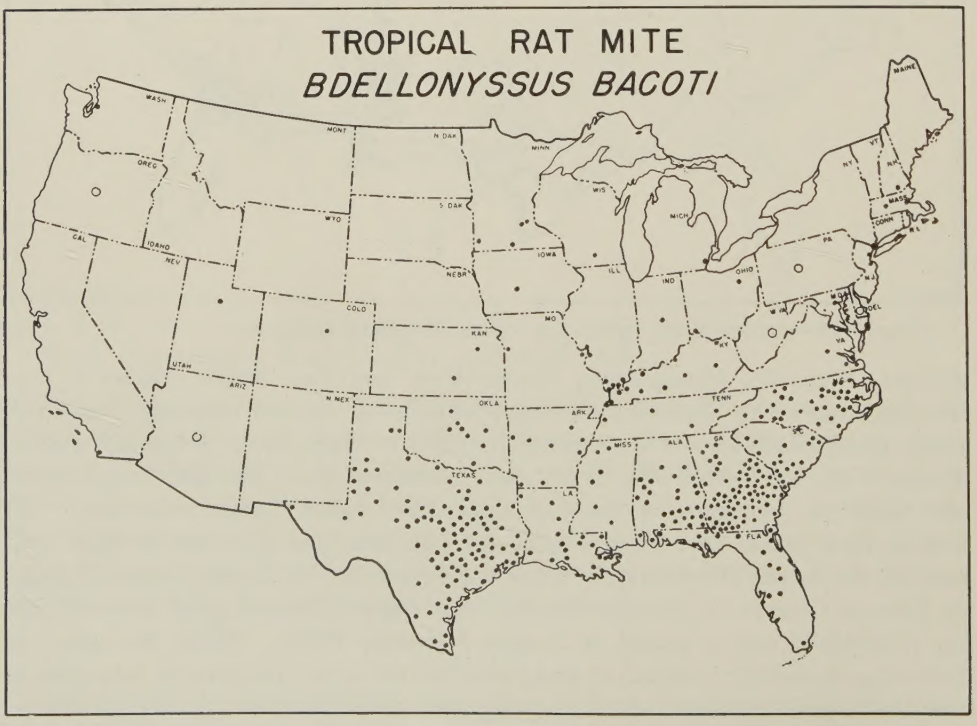

FIG. 9. Distribution of the Tropical Rat Mite (*Bdellonyssus bacoti*).

RAT MITES

Four species of mites have been found commonly on domestic rats: the tropical rat mite, *Bdellonyssus bacoti* (Hirst), the spiny rat mite, *Laelaps echidninus* Berlese, the domestic rat mite, *Laelaps nuttalli* Hirst, and the common rodent mite, *Haemolaelaps glasgowi* (Ewing). The first three of these probably were introduced into the United States on domestic rats while the common rodent mite probably is a native species which has become relatively abundant on domestic rats in the northern part of this country.

The map showing the distribution of the tropical rat mite (*B. bacoti*) (Fig. 9) makes one fact very evident, i.e. that the name, tropical rat mite, is a misnomer. Specimens have been collected all across the northern part of United States, from

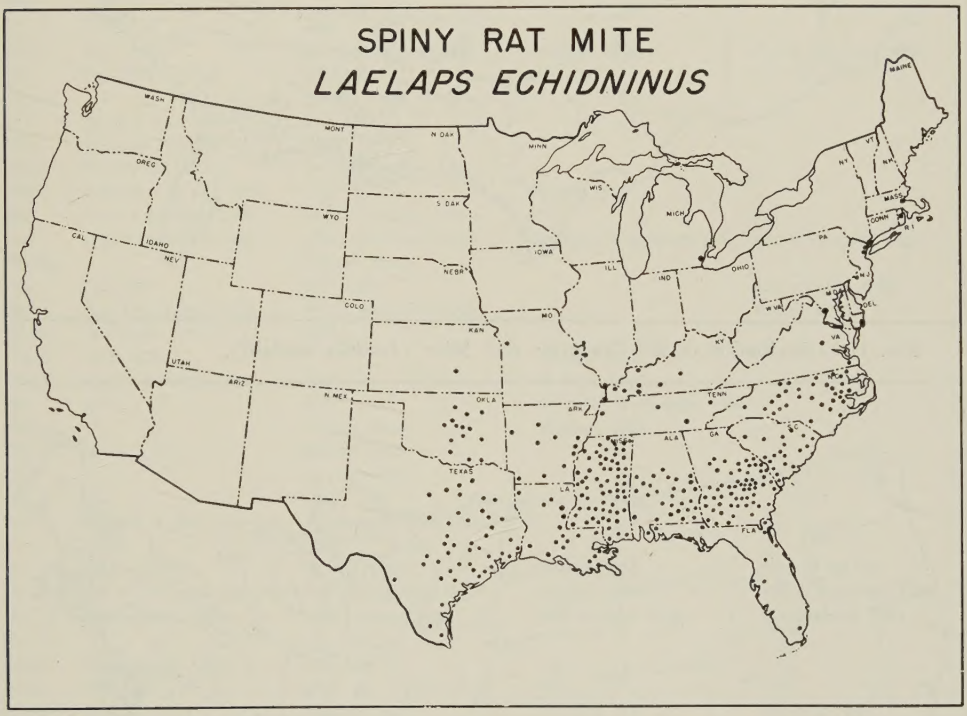

FIG. 10. Distribution of the Spiny Rat Mite (*Laelaps echidninus*).

Massachusetts, New Hampshire, New York, and New Jersey, across Michigan and Iowa to San Francisco, Calif., and Seattle, Wash. It is, however, much more abundant in the southern part of the United States than in the northern portion. The tropical rat mite is the only one of the four common rat mites which frequently deserts domestic rats to bite man or his domestic and laboratory animals. Numerous collections of tropical rat mites which have bitten man, causing extreme discomfort and skin irritations or rashes, have been received at the Communicable Disease Center from all parts of the United States and even from Alaska. (See Bishopp, 1923; Shelmire and Dove, 1931; and Olson and Dahms, 1946).

The spiny rat mite (*L. echidninus*) (Fig. 10) and the domestic rat mite (*L. nuttalli*) (Fig. 11) have a wide distribution in eastern United States and may

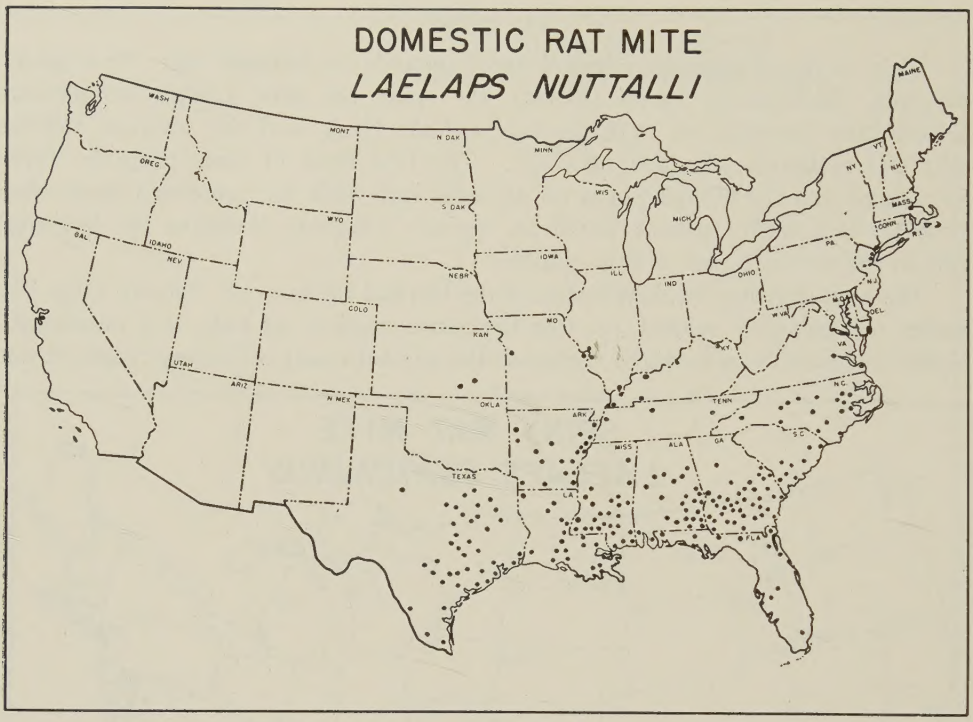

FIG. 11. Distribution of the Domestic Rat Mite (*Laelaps nuttalli*).

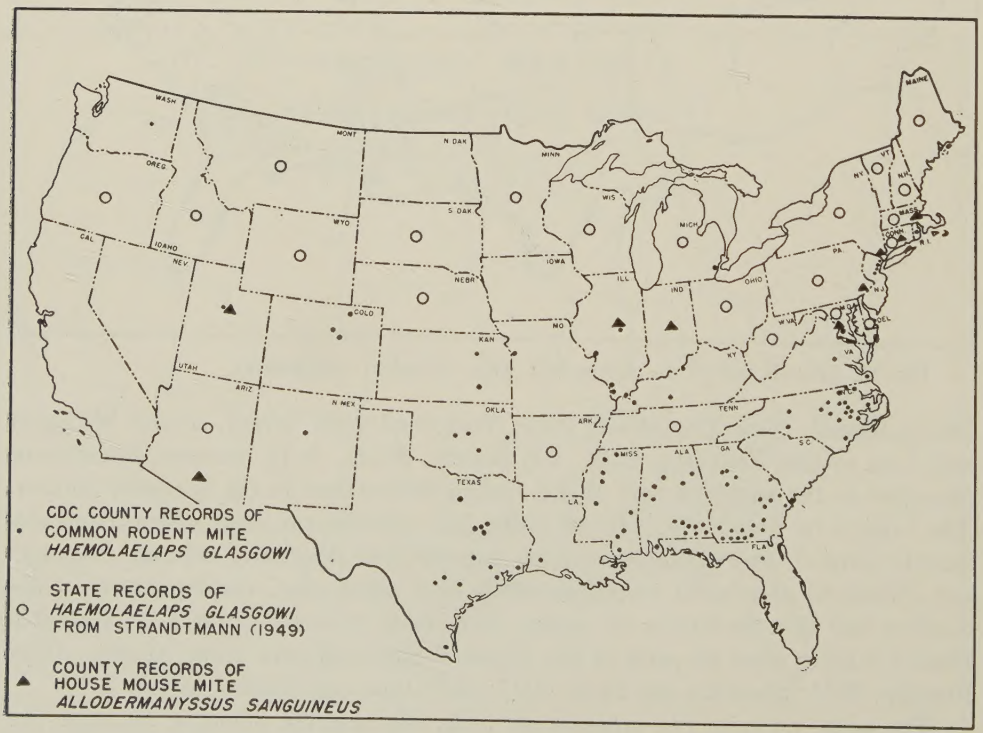

FIG. 12. Distribution of the Common Rodent Mite (*Haemolaelaps glasgowi*) and the House Mouse Mite (*Allodermanyssus sanguineus*).

be found at the larger seaports on the Pacific Coast. These two species, which have a tropicopolitan distribution, seem to be specific parasites of domestic rats.

The common rodent mite (*H. glasgowi*) (Fig. 12) is a native species which

Table I

STATE RECORDS OF FOUR COMMON SPECIES OF DOMESTIC RAT FLEAS IN THE UNITED STATES

State	*Xenopsylla cheopis*	*Nosopsyllus fasciatus*	*Echidnophaga gallinacea*	*Leptopsylla segnis*
Ala.	Cole & Koepke 1946	Cole & Koepke 1946	Fox 1940	Cole & Koepke 1946
Ariz.	Prince 1943		Trembley & Bishopp 1940	
Ark.	CDC	CDC	Fox 1940	CDC
Calif.	Fox 1908	Fox 1908	Mitzmain 1909	Fox 1908
Colo.	Prince 1943	Prince 1943		CDC
Conn.				
Del.				
D. C.	Fox 1940	CDC		
Fla.	Fox 1940	Fox & Sullivan 1925	Packard 1894	Fox & Sullivan 1925
Ga.	Fox 1931	Fox 1931	Fox 1931	Fox 1931
Idaho		Prince 1943		
Ill.	Ewing & Fox 1938	Fox 1940		
Ind.	Wallace 1925			
Iowa	Roudabush 1939	Osborn 1896		
Kans.	Grundman *et al.* 1941	CDC	Parman 1926	
Ky.	Good & Kotcher 1949	Good & Kotcher 1949	CDC	CDC
La.	Fox & Sullivan 1925	Fox & Sullivan 1925	Trembley & Bishopp 1940	Fox & Sullivan 1925
Maine		Fuller 1943		
Md.	Fox 1940	Fox 1940		Yeh & Davis 1950
Mass.	Fox & Sullivan 1925	Fox & Sullivan 1925		Fuller 1943
Mich.	Jachowski 1943	CDC		
Minn.	Owen 1936		Parman 1926	
Miss.	Gray & Kotcher 1947	CDC	Herrick 1908	
Mo.	CDC	CDC	Trembley & Bishopp 1940	
Mont.		Prince 1943	Stewart 1932	
Nebr.	Gates 1944	Prince 1943		
Nev.		Prince 1943		
N. H.	CDC	CDC		
N. J.	Hansens & Hadjinicolaou 1952	Hansens & Hadjinicolaou 1952		
N. Mex.	Prince 1943	Prince 1943	Prince 1943	Prince 1943
N. Y.	Fox & Sullivan 1925	Fox & Sullivan 1925	Stewart 1928	Fox & Sullivan 1925
N. C.	Shaftesbury 1934	Shaftesbury 1934	Shaftesbury 1934	Shaftesbury 1934
N. D.				
Ohio	Ewing & Fox 1938	Fox 1940		
Okla.	CDC	CDC	CDC	CDC
Oreg.	Hubbard 1947	Hubbard 1947	Hubbard 1947	Hubbard 1947
Pa.	Vogel & Cadwallader 1935	Vogel & Cadwallader 1935	Vogel & Cadwallader 1935	Vogel & Cadwallader 1
R. I.	Robinson 1913	Robinson 1913	Trembley & Bishopp 1940	Robinson 1913
S. C.	CDC	CDC	Fox 1940	Fox 1940
S. Dak.				
Tenn.	Trembley & Bishopp 1940	CDC	Trembley & Bishopp 1940	CDC
Tex.	Kemp 1931	Kemp 1931	Trembley & Bishopp 1940	Randolph & Wade 1946
Utah	Prince 1943	Prince 1943	Tipton & Allred 1951	
Vt.				
Va.	Hasseltine 1929	Hasseltine 1929	Hasseltine 1929	Hasseltine 19
Wash.	Fricks 1936	Fricks 1936		Fricks 1936
W. Va.				
Wis.	Schiller & Morgan 1949	Fox 1940		
Wyo.	Wiseman 1950	Prince 1943		

is found on a wide variety of native hosts in the United States. Records of Strandtmann (1949) and data available in the Communicable Disease Center indicate that this mite is found throughout the United States. At the present time,

definite records are available from all the states in this country except Indiana, Iowa, North Dakota, and Nevada. In the southern part of the United States, *B. bacoti, L. echidninus,* and *L. nuttalli* are usually the most abundant species of rat mites, but in the northern United States, *H. glasgowi* is often by far the most abundant rat mite. Knutson and Szymkowicz (1952), for example, found in a

Table II

STATE RECORDS OF TWO COMMON SPECIES OF DOMESTIC RAT LICE IN THE UNITED STATES

State	*Polyplax spinulosa*	*Hoplopleura oenomydis*
Ala.	Cole & Koepke 1946	Pritchard 1947
Ariz.		
Ark.	CDC	CDC
Calif.	Kellogg & Ferris 1915	CDC
Colo.	CDC	
Conn.	Osborn 1923	
Del.		
D. C.	CDC	
Fla.	Rumreich & Wynn 1945	Pritchard 1947
Ga.	Fox 1931	Pritchard 1947
Idaho		
Ill.	CDC	
Ind.		
Iowa	Osborn 1891	Roudabush 1939
Kans.	CDC	
Ky.	Good & Kotcher 1949	
La.	CDC	CDC
Maine		
Md.		
Mass.	Fox & Sullivan 1925	
Mich.	CDC	
Minn.		
Miss.	CDC	
Mo.	CDC	CDC
Mont.		
Nebr.		
Nev.		
N. H.	CDC	
N. J.	CDC	
N. Mex.		
N. Y.	Fox & Sullivan 1925	
N. C.	Harkema 1936	Schoof 1948
N. D.		
Ohio	CDC	
Okla.	CDC	
Oreg.		
Pa.		
R. I.	Robinson 1913	
S. C.	CDC	
S. Dak.		
Tenn.	CDC	Pritchard 1947
Tex.	Randolph & Eads 1946	Pritchard 1947
Utah	CDC	
Vt.		
Va.	Hasseltine 1929	CDC
Wash.		CDC
W. Va.		
Wis.		
Wyo.	CDC	

small Rhode Island town that 93 percent of the mites collected on rats were the common rodent mite.

The house mouse mite (*Allodermanyssus sanguineus,* Hirst) (Fig. 12) was first collected in Washington, D. C. in 1909. Apparently no other specimens were collected anywhere in the United States until 1938, but since that year it has been reported from Boston, New York, Philadelphia, Washington, Indianapolis, and

Tucson, Ariz. In 1946, this mite received considerable attention due to the discovery of Huebner, Jellison, Pomerantz, and their co-workers that *Alloder-*

Table III

STATE RECORDS OF FOUR COMMON SPECIES OF DOMESTIC RAT MITES IN THE UNITED STATES

State	*Bdellonyssus bacoti*	*Haemolaelaps glasgowi*	*Laelaps echidninus*	*Laelaps nuttalli*
Ala.	Cole & Koepke 1946	Cole & Koepke 1946	Cole & Koepke 1946	Cole & Koepke 1946
Ariz.	Williams 1946	Strandtmann 1949		
Ark.	Williams 1946	Strandtmann 1949	CDC	CDC
Calif.	Ewing 1932	Ewing 1925	CDC	CDC
Colo.	CDC	Strandtmann 1949		
Conn.		Strandtmann 1949		
Del.	Williams 1946	Strandtmann 1949		
D. C.	Ewing 1923	CDC	CDC	
Fla.	Ewing 1932	Worth 1950	Rumreich & Wynn 1945	Rumreich & Wynn 1945
Ga.	Ewing 1932	Cole & Koepke 1947	Fox 1931	Fox 1931
Idaho		Strandtmann 1949		
Ill.	McIntosh 1935	CDC	CDC	CDC
Ind.	CDC			
Iowa	Roudabush 1939			
Kans.	Hungerford 1943	Strandtmann 1949	CDC	CDC
Ky.	Good & Kotcher 1949	Good & Kotcher 1949	Good & Kotcher 1949	CDC
La.	Ewing 1932	CDC		CDC
Maine		Strandtmann 1949		
Md.	Ewing 1932	Strandtmann 1949		
Mass.	Ewing 1932	Strandtmann 1949	Fox & Sullivan 1925	Fox & Sullivan 1925
Mich.	Williams 1946	CDC	CDC	
Minn.	Riley 1940	Strandtmann 1949		
Miss.	Ewing 1923	Strandtmann 1949	CDC	CDC
Mo.	Ewing 1923	CDC	CDC	CDC
Mont.		Strandtmann 1949		
Nebr.		Strandtmann 1949		
Nev.				
N. H.	Williams 1946	Strandtmann 1949		
N. J.	Lowell 1946	Strandtmann 1949	CDC	CDC
N. Mex.		CDC		
N. Y.	Ewing 1923	Strandtmann 1949	Fox & Sullivan 1925	Fox & Sullivan 1925
N. C.	Ewing 1932	Harkema 1936	Harkema 1936	Schoof 1948
N. D.				
Ohio	Ewing 1932	Strandtmann 1949		
Okla.	Williams 1946	CDC	CDC	
Oreg.	Williams 1946	Strandtmann 1949		
Pa.	Williams 1946	Strandtmann 1949		
R. I.		Knutson & Szymkowicz 1952	Robinson 1913	
S. C.	Williams 1946	Strandtmann 1949	CDC	
S. D.		Strandtmann 1949		
Tenn.	Ewing 1932	Strandtmann 1949	CDC	CDC
Tex.	Bishopp 1923	Randolph & Eads 1946	Randolph & Eads 1946	Randolph & Eads 1946
Utah	CDC	Strandtmann 1949		
Vt.		Strandtmann 1949		
Va.	Hasseltine 1929	Ewing 1925	Hasseltine 1929	Hasseltine 1929
Wash.	Ewing 1932	Strandtmann 1949		
W. Va.	Williams 1946	Strandtmann 1949		
Wis.	Williams 1946	Strandtmann 1949		
Wyo.		Strandtmann 1949		

manyssus was a vector of rickettsialpox from mice to man in New York City (Huebner, *et. al.* 1946). Subsequently, Pratt, *et al.* (1949) added Champaign, Ill., and Salt Lake City, Utah, to the distribution map of this species. The Utah

specimens are particularly interesting since they were collected on Norway rats, whereas the normal host undoubtedly is the house mouse. The most recent new locality where this mite has been collected is West Hartford, Conn., in 1952. There, the house mouse mite was collected on mice in apartment houses and near incinerators during a rickettsialpox epidemic, under conditions similar to the classic New York outbreak of 1946 (Rindge, 1952).

During the course of this study the writers have accumulated numerous records from the literature relating to the distribution of rat ectoparasites in the United States. In tables I, II, and III there is listed one state record for each species, either from the literature or from Communicable Disease Center sources, which appears to be reliable. This is not always the first record of a species from the particular state. Thus, in table I, the Alabama record of *X. cheopis* was published by Cole and Koepke (1946). Similarly, the New Hampshire record of *X. cheopis* is based on specimens from Manchester, N. H., determined at the Communicable Disease Center and listed as "CDC."

The absence of a specific record does not necessarily mean that the species does not occur in the state, but merely that no published record has been found, or that no collection of data on rat ectoparasites of the state has been made available to the writers.

SUMMARY

As a result of the cooperative murine typhus control programs of the State health departments and the Communicable Disease Center, a large amount of new data has become available on the general geographical distribution of a number of common domestic rat ectoparasites. Some of these ectoparasites are known or suspected vectors of both murine typhus and plague, from rat to rat, or from rat to man.

The geographical distribution of these common rat ectoparasites is discussed and reference is made to spot maps showing the occurrence of the following: four common rat fleas, *Xenopsylla cheopis, Leptopsylla segnis, Nosopsyllus fasciatus,* and *Echidnophaga gallinacea;* two common rat lice, *Polyplax spinulosa* and *Hoplopleura oenomydis;* and four common rat mites, *Bdellonyssus bacoti, Laelaps echidninus, Laelaps nuttalli,* and *Haemolaelaps glasgowi.* In addition data are presented on the house mouse mite, *Allodermanyssus sanguineus,* vector of rickettsialpox.

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STUDIES ON THE CHEMOTHERAPY OF EXPERIMENTAL SCHISTOSOMIASIS. I. A METHOD FOR DETECTING SCHISTOSOMACIDAL ACTIVITY BASED ON RESPONSE OF *SCHISTOSOMA MANSONI* INFECTIONS IN MICE TO FUADIN THERAPY*

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At least three techniques have been reported to be of value in the search for compounds active against mature infections of *Schistosoma mansoni* in mice. First, the method of Kikuth *et al.* (1946, 1948) by which the activity of Miracil was discovered, is based on whether the administration of the test compound starting the forty-eighth day of infection causes the disappearance of the eggs of this parasite from the feces for 3 weeks. However, this procedure has the disadvantage that the schistosome eggs may not appear in the feces every day and usually it is necessary to use concentration methods before they can be found by microscopic examination of fecal matter.

The second type of technique is one described by Schubert (1948a) and also employed by others. About 8 weeks after exposure to *S. mansoni,* mice are treated. After a rest period the animals are examined for worms by a careful dissection or by time-consuming perfusion of the portal system and liver. If there is a significant reduction in the number of worms and they are found mostly in the liver, this is evidence of activity. However, because of the great variation in the number of worms resulting from a given exposure of cercariae and because of the complicated procedure involved, this method leaves much to be desired. None the less, by use of this method the value of the oral administration of certain thio-antimonials was demonstrated by Schubert (1948b).

A third procedure for detecting the anti-schistosome properties of compounds was developed by Bueding *et al.* (1947, 1951, 1953). These workers found that adult *S. mansoni* removed from animals which had been treated with a cyanine dye had a markedly depressed respiration. However, no permanent effects on this parasite resulted from the treatment of infected mice with the dye, thus indicating that the parasites can live by anaerobic metabolism. There was a significantly reduced glycolysis in schistosomes removed from animals given 2-methyl-1, 4-naphthoquinone and the authors found that these parasites were more sensitive to this physiological change than to the inhibition of respiration. Thus the authors set up an anti-glycolytic index as a measure of schistosomacidal activity of compounds tested. The method of determining the anti-glycolytic index is somewhat complicated and there remains the question whether the schistosomes behave the same physiologically *in vitro* when removed from the host as they would *in vivo* after exposure to each test compound.

Because of the disadvantages of the described screening techniques, it was of interest to investigate the possibility of a more practical procedure for detecting

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schistosomacidal activity *in vivo*. The results of the present study of the effects of sodium antimony biscatechol disulfonate (Fuadin) in mice given lethal infections of *S. mansoni* suggest another approach to the rapid recognition of activity against the mature schistosome infection. First, a compound is schistosomacidal if it increases the survival time of infected mice. Second, activity is confirmed by the finding of dead worms in the liver, usually in lesions larger but fewer than the egg-containing lesions typical of the untreated infection. These conditions were observed consistently when mice were given adequate early treatment with Fuadin or with other active drugs and thus they serve as the basis for the method to be outlined.

MATERIALS AND METHODS

The animals used were weanling female mice of a Swiss albino strain which was started from registered stock at the National Institutes of Health in 1936. The animals weighed 12 to 18 gm. and were exposed to infection by the tail immersion technique (Wright *et al.*, 1948; Olivier and Stirewalt, 1952). The cercariae used were of a Puerto Rican strain of *S. mansoni* maintained in this laboratory since 1943 (Cram, 1947; Files and Cram, 1949). The intermediate host, *Australorbis glabratus*, of Puerto Rican origin was raised by the Section on Helminthic Diseases of this laboratory and the snails were supplied when they were from 5 to 10 mm. in diameter. They were exposed en masse to miracidia and the usual method of obtaining schistosome cercariae from the infected snails was followed. Cercariae from several lots of *A. glabratus* were combined and the concentration of cercariae in the harvest was estimated by counting the number present in three aliquots of the suspension, and determining the number per milliliter. The volume of the suspension containing the desired number of cercariae was placed in Kahn serum tubes by use of a 3 or 5 ml. automatic pipette made by the Alfred Bicknell Co. If necessary, water was added to fill the tubes within one centimeter of the top. Shortly before exposure the mice were given an intraperitoneal injection of Veterinary Nembutal, 81 mg. pentobarbital sodium per kilogram body weight, which was sufficient to immobilize them for the exposure period of 30 minutes. Ten animals were placed in supine position on a wooden block in a fashion similar to that described by Olivier and Stirewalt (1952) except that it was unnecessary to first fasten the anesthetized mice on small individual boards. A rubber band stretched lightly over the thorax and another over the lower abdomen held the mice, while their tails were suspended in the Kahn tubes. As an added precaution a small notched rubber stopper was employed to hold the tails of the mice in the tubes. By this method two operators could expose approximately 200 mice to cercariae during a three-hour period.

After exposure the mice were housed in holding cages in groups of 50. They were fed Purina Chow, were given fresh water daily, and their mortality was recorded. Thirty-five or forty-two days after exposure the animals were divided into groups of 5 or 10 individuals for treatment and each member of the groups was given intraperitoneal or oral injections of drug twice daily for 5 or 10 days, or was retained as an infected, non-treated control. The condition of the mice was noted daily; the body weight was recorded at the beginning and end of the treatment period, and also at the time of autopsy. Mice dying toward the completion of treatment or thereafter were autopsied and the survivors were killed 8 weeks after the

date of exposure. Upon autopsy of each animal the type of lesions and their extent on the ventral surface of the median lobe of the liver or that part of the organ first seen upon opening the abdomen was used to grade the gross liver pathology as follows: 0, no lesions; 1+, very few lesions, i.e. 1 to 10 small scattered egg lesions and/or 1 to 5 larger dead worm lesions on the outer periphery of the lobe; 2+, about one quarter of the liver lobe involved with egg lesions or dead worm lesions; 3+, roughly about 50 per cent of the lobe occupied by egg-lesions, with little pigment present; and 4+, practically all of the organ occupied by egg-granulomata, pigment present, and marked enlargement of the liver. Samples of the liver were examined microscopically as press-preparations, which are described later. In addition to the gross examination of the liver and portal system, this organ and system of many of the animals were perfused by the method of Yolles *et al.* (1947). The number, location, and condition of the worms were observed. After perfusion, samples of the liver 1 cm. by 0.5 cm. by 0.5 cm. were pressed between two glass slides (7 cm. by 15 cm.) and were examined under the dissecting microscope for the presence of eggs and live or dead worms. The content of some of the lesions seen on the livers of the animals was determined by the examination of the press preparations. The whole livers of a few of the mice were placed in 20 ml. of physiological salt solution and were macerated in a Waring Blendor for two minutes. Thirty milliliters of the saline solution were added and the suspension was blended for 30 seconds. The total number of eggs in each liver was estimated from the average count of 3 aliquots (2 ml. each) of the liver suspension which had been filtered through one thickness of surgical gauze. In some of the series, groups of animals were kept as non-infected but treated controls. These were weighed and observed for toxicity and pathology as were the others of the series.

RESULTS

Mortality of Mice Exposed to Various Numbers of S. mansoni *Cercariae.*

Eight series of mice weighing an average of 13 to 16 gm. each, were divided into groups of 10 or 20 and were exposed to 250, 350, 450, or 900 cercariae per animal. The mice survived an average of 8 to 9 weeks after exposure to 250 cercariae, 7 weeks after exposure to 350 to 450 cercariae, and 5 weeks after 900 cercariae.

It was found that the mortality rate in 21 series of mice varied with the size of exposure. When 20 to 25 cercariae were given per gram of body weight, about 5 per cent of the mice died during the first five weeks. The exposure to 20 cercariae per gram killed 67 per cent of the animals during the period from the sixth through the eighth week, while 22 to 25 cercariae per gram killed 88 per cent during this same period. When the mice were exposed to 30 or 35 cercariae per gram body weight, about 15 per cent of the mice died in the first five weeks and 72 to 79 per cent, respectively, died in the next three weeks. Thus exposures of from 22 to 25 cercariae per gram body weight were fatal for the greatest percentage of young mice from the sixth through the eighth week after exposure. During this period the paired schistosomes were beginning to lay eggs which resulted in great destruction of liver and intestinal tissue.

Response of Infections to Fuadin Therapy.

Since a critical period for mice was after the thirty-fifth day of infection, it was of interest to see if Fuadin therapy started at that time would prevent deaths of the

animals. A comparison was made of the response of 3 groups of 10 infected mice, each to different regimens of the drug. One group received 125 mg. Fuadin/kg. intraperitoneally twice daily for 5 days, starting the thirty-fifth day of infection; a second was started on the same regimen the forty-second day, while the third lot served as an untreated control. This series was repeated three times. Fuadin treatment undertaken the thirty-fifth day of infection prevented the death of most of the mice for two weeks after treatment. However, when therapy was delayed one week, most of the animals succumbed as did the non-treated controls. Animals treated earlier could easily be recognized because of their better physical appearance.

In all the series the gross and microscopic appearance of the liver depended on whether the animal had been treated and on what day treatment was started. The livers of the majority of those placed on the earlier treatment contained a few small pseudotubercles but almost all had larger lesions and pigment mostly along the outermost periphery. The average grade of liver lesions for the animals started on treatment day 35 of the infection was 1.6+. Upon examination of simple press preparations made of small samples from various areas of the liver from these mice, dead or dying worms were easily recognized in the larger lesions while schistosome

TABLE 1.—*The effects of graded doses of Fuadin given twice daily for 10 days against* S. mansoni *in white mice exposed to 450 cercariae each*

No. Mice	Daily Dose Fuadin (Start Day 35) mg./kg.	No. Mice Dying (Day 35 to Day 56)	Autopsy Results		
			Av. No. Worms/Mouse	Av. No. Eggs/Liver	Av. Grade Liver Lesions
10	250.0	0	0.1	0	1+
10	125.0	0	0.4	0	1+
9	62.0	0	16.0	VF*	1+
9	31.0	0	68.0	1,465	2+
9	15.5	0	84.0	1,804	2+
8	Controls	7	100.0	6,776	4+

* VF = relatively very few in number.

eggs were present in the relatively scarce pseudotubercles. By comparing the average number of live worms recovered by perfusion from mice of this treated group with the average number recovered from the untreated controls, it was estimated that 85 per cent of the worms were killed by this early treatment. No worms were seen in the hepatic portal vein at its entrance into the liver, but many were in this location in the controls.

Egg-lesions were scattered throughout the livers of the majority of those started on treatment day 42 of infection and there was diffuse pigment in some. Few larger lesions containing dead worms were detectable by gross examination or by press preparation. The average grade of liver lesions for this group was 3+. Though 83 per cent of the worms were killed by the treatment, so much damage had been done to the host that the treatment was insufficient to prevent death of the animals.

No large lesions or dead worms were seen in the livers of the untreated controls, which contained numerous egg lesions and heavy diffuse pigment. The average grade of liver lesions of the controls was 3+.

Since it was found that an optimum time to start the Fuadin treatment of mice was the thirty-fifth day after exposure to a lethal dose of *S. mansoni* cercariae, a group of 55 mice infected for 5 weeks and a group of 60 non-infected controls were each divided into 6 lots. Each lot of 9 or 10 mice was placed on a different dosage

of Fuadin; these ranged from 15.5 mg./kg. to 250 mg./kg. (Table 1). These doses were given twice daily for 5 days, and after rest of two days the mice were treated for another 5 days. One lot of animals was kept as a non-treated control and one lot as a non-infected but treated control. The results of examining the treated and untreated animals are summarized in Table 1. It can be seen in the table that during the first 8 weeks of infection death was prevented by even the lowest dose of Fuadin. Most of the animals given either 250 or 125 mg. Fuadin/kg. were cleared of living worms, while the remainder harbored only a few degenerate schistosomes and these were found in the liver for the most part. The two highest doses suppressed egg-laying completely and dead worms were found in characteristic liver lesions, with the average grade of liver lesions being 1+. Dead worm lesions also resulted from the 62 mg. Fuadin/kg. treatment which brought about a reduction of more than 80 per cent of the worm burden (cf. Table 1). Although some live worms were present, there were very few eggs, and the liver pathology was 1+. These eggs may have been laid about the time treatment started since a few eggs have been found by the thirty-fifth day of infection. Likewise, there were very few or no eggs in the intestinal wall of these mice. The animals treated with 31 or 15.5 mg./kg. harbored numerous live worms which had been adversely affected since the egg counts were significantly reduced, but the liver pathology was 2+. Dead worms were not found in the livers of the mice receiving the lowest dose of drug nor in the controls. The grade of the liver lesions of the controls was 4+. Although it was difficult to distinguish between the grade of liver pathology found in those animals treated with either of the two highest doses or either of the two lowest doses, it was easy to distinguish the livers of the animals given the highest from those given the lowest doses, and those of the untreated controls. The larger lesions containing dead worms were characteristic of the livers of the groups given the higher doses while the small egg-containing pseudotubercles were the only lesions visible in the livers of those given the lowest doses, or in the controls.

Two other series of 48 and 54 animals each were treated in the same fashion and with results similar to those described for the first series with the exception that the average worm- and egg-burden were somewhat larger. More of the untreated mice died and greater numbers of dead worm lesions were observed in the treated mice. Three of 20 animals in another series which were given 250 mg. Fuadin/kg. died, thus this dose was about the maximum tolerated level.

In view of the fact that 125 mg. Fuadin/kg. was the minimum curative dose and also because it was tolerated, it was selected as the standard dose of Fuadin to be utilized in all subsequent tests.

Since the administration of 125 mg. Fuadin/kg. twice daily (b.i.d.) to mice for either 5 or 10 days, starting the thirty-fifth day of infection, resulted in large lesions containing dead worms in most animals treated, it was of interest to compare the infections in mice 2 weeks after some had been subjected to each of these two regimens. Accordingly, 10 mice were treated with 125 mg./kg. b.i.d. for 5 days and 10 were given the same dosage for 10 days. Compared with 10 untreated controls, in two series it was found that the 5-day regimen reduced the average infection from 97 to 16 live schistosomes in one, and from 89.7 to 15.6 in the other; while the longer regimen reduced the average infection to 1 worm or less. Nevertheless, the 5-day treatment prevented death of the mice for 8 weeks and produced hepatic le-

sions containing dying or dead worms. These were chosen as satisfactory criteria for the schistosomacidal activity of Fuadin because of their constancy and the ease with which they could be determined.

Screening Technique.

A period of 8 weeks was determined to be the minimum time necessary to run a test for drug activity against mature *S. mansoni* infections in mice because of the following. The majority of the schistosomes were observed to be paired at the end of the fifth week of infection. As was shown above, schistosomacidal activity of Fuadin was easily demonstrated when the drug was given twice daily for five consecutive days, starting on the thirty-fifth day after exposure. Furthermore, as was observed by Schubert (1948a), treatment with active drugs caused the worms to migrate to the liver and permanent effects on the parasites could not be determined before the second week after treatment. Thus an evaluation of drug effects on mature *S. mansoni in vivo* could not be made before 8 weeks had elapsed.

Since exposures of 22 to 25 cercariae/gm. body weight were lethal for most young mice within 7 to 8 weeks, groups of 100 to 200 mice four or five weeks old were exposed to a dose in or near this range. It was found that mice weighing about 15 gm. or more gave the best results. Thirty-five days later the lot was divided into groups of 5 mice each. The members of each group were given the previously determined maximum tolerated dose of the test drug twice daily for 5 days. The maximum tolerated dose is approximately that which could be given for 5 days b.i.d. without causing loss of weight. With each test series two control groups were included: (1) a group of 10 untreated controls and (2) a group of 5 treated with Fuadin at 125 mg./kg. twice daily for 5 days.

Mortality was recorded daily through 8 weeks of infection. Animals dying after treatment as well as the survivors were autopsied to determine the status of the infection. Upon opening the abdomen the hepatic portal vein was examined. If numerous large schistosomes could be seen in the portal vein just before its entrance into the liver, this indicated that the compound given was without schistosomacidal power. The livers of all mice were examined grossly for type of lesions present and these were graded 0, 1+, 2+, 3+, or 4+, depending on the extent that the median lobe was involved. Press preparations of several samples of the liver were quickly examined under the low power of the dissecting scope in order to determine the presence and relative numbers of living or dead worms or eggs.

Performance of Technique.

Several compounds have been tested by the procedure described, and some of the results are summarized in Table 2. Screening tests of the first three compounds and of compound 6 have not been reported previously, while the remaining three are known to have chemotherapeutic value in human or experimental schistosomiasis. It can be seen in the table that treatment with compound 1 resulted in no greater survival of mice than in the untreated control group; however, the percentage of mice with dead worms in the liver appeared to be greater in the treated group. Compound 2 showed slight activity as evidenced by the increased percentage of mice surviving and by the greater percentage of mice with dead worms in the liver. The average grade of liver pathology for this compound was about the same as

that of the untreated controls. Compounds 3 and 4 exhibited moderate activity in that they prevented death of about 45 per cent of the mice in the test period and dead worms were found in 48 per cent or more of the animals, but the average grade of liver pathology was not significantly reduced. Compounds 5, 6, and 7 were found to have a high degree of activity by all three criteria-high percentage survival, high percentage of animals with dead worms, and minimal liver pathology. However, the administration of compound 6 resulted in a significantly lower percentage of mice with dead worms, and thus a lesser degree of activity, than did compounds 5 and 7. That the technique did detect various grades of schistosomacidal activity was confirmed by the results of perfusing some of the mice treated with compounds 2, 4, 5, 6, and 7. The number of live worms recovered was inversely proportional to the grade of activity ascribed the compound by the present technique.

TABLE 2.—*Results of screening method with various compounds administered intraperitoneally twice daily for 5 consecutive days to mice exposed to lethal infections of* S. mansoni

Compound	No. Mice	Percentage of Mice		Av. Grade Liver Pathology
		Surviving 8 weeks	With dead worms in liver	
1. 9-(Cyanoacetamido)-xanthene	20	5.0	6.6	2.4+
2. 1-(*p*-Hydroxyphenyl)-2-thiourea	40	12.5	12.1	3.5+
3. 9-(*a*-Toluenesulfonamido)-xanthene	15	46.6	69.2	2.7+
4. Miracil D*	34	44.1	47.8	3.0+
5. *n*-Butyl-3,4-stibogallate**	15	86.6	100.0	1.4+
6. Friedheim TWSb-K***	28	96.4	78.2	1.1+
7. Fuadin	104	93.2	97.8	1.5+
Controls	248	4.8	0.9	3.4+

* The author wishes to thank the Chemical-Biological Co-ordination Center for the supply of Miracil D (1-(2-diethylaminoethylamino)-4-methyl-10-thiaxanthone hydrochloride).
** The author wishes to thank E. R. Squibb & Sons for the *n*-butyl-3,4-stibogallate used in this study.
*** Monopotassium antimony (III) *a*-*a'*-dimercaptosuccinate from Dr. Ernst A. H. Friedheim who will report on its activity in a forthcoming paper.

DISCUSSION

Since the activity of the above compounds was detected by the procedure described, the technique is thus thought to be suitable for preliminary assay of anti-schistosome powers of compounds. The advantages of this technique over the previously described methods of screening drugs for schistosomacidal activity are several. First, 35 days after exposure to lethal doses of cercariae, the mice are started on an intensive treatment. An active drug will prevent death of the mice by killing off the worms before they can lay large numbers of eggs and cause extensive damage to the liver and intestine. The efficacy of the treatment can thus be quickly ascertained by observation of the mortality of the animals and of the gross pathology of the liver, as well as the presence of worms in the hepatic portal or mesenteric vessels.

In a recent paper by Bueding *et al.* (1953), it was shown that the schistosomes are more susceptible to activity of cyanine dyes when treatment was started on day 27 or 30 of infection instead of on day 49. This also may be the case with other active compounds as was shown for Fuadin in the present study. If this is true, then the initiation of treatment early not only facilitates the reading of results in the manner described, but also is a more sensitive procedure for the detection of anti-schistosome activity.

It is proposed that the method outlined be utilized as a preliminary step to discover whether or not a compound is schistosomacidal, and, if so, its relative degree of activity. Any compound with activity falling between that of Miracil D and Fuadin at its maximum tolerated dose would be studied in detail. A therapeutic index based on the dose of the compound lethal for 50 per cent of the animals and the dose which would clear the infection from 50 per cent of the animals would be determined. If the resulting therapeutic index compared favorably with that of Fuadin, the activity of the compound in question would be investigated in mice which 8 weeks before had each been exposed to 200 cercariae of *S. mansoni.*

SUMMARY

1. A simplified method of screening compounds for schistosomacidal activity has been developed based on the response of young mice given potentially lethal infections of *Schistosoma mansoni* to therapy with Fuadin.

2. Increased survival time of treated animals and the presence of dying or dead *S. mansoni* in their livers were shown to be reliable criteria of activity.

3. The efficiency of the described screening technique was confirmed by the detection of the activity of additional compounds which have chemotherapeutic value in human or experimental schistosomiasis.

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NOTES ON THE OCCURRENCE AND BIOLOGY OF FILARIAL NEMATODES IN SOUTHWESTERN GEORGIA

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Early in 1952, a comprehensive study of filarial nematodes and their arthropod vectors was begun in this laboratory, with the objective of investigating basic factors involved in transmission of these parasites. A survey of the filarial fauna in a limited part of southwestern Georgia was conducted to determine the availability of local filariae for experimental work.

Collection of vertebrate species was carried on from February 1 to October 15, 1952. Fresh blood-saline preparations from the heart and lungs of animals and stained films of the same type were examined for microfilariae. Smears of other organs sometimes were made. Mosquitoes were used occasionally for xenodiagnosis in living animals. Filarioid eggs were not sought specifically in the smears until near the end of the survey, but it is believed that any eggs present usually were detected during the search for microfilariae, or during later study of stained slides. An intensive effort was made to find adult worms in about 20 per cent of the infected specimens. The technique used was to dissect the animal, sometimes into minute fragments, then immerse the parts for one to three hours in saline heated to 37 degrees Centigrade. The mixture was agitated and sedimented and the sediment then examined microscopically.

Information concerning infected hosts, occurrence, distribution, and observations on the biology of the worms are given in this report. Taxonomic, morphologic, and life cycle studies will be presented in other reports.

FINDINGS

The species studied and the number of individuals examined and found infected are given in Table 1. A similar list of species not found infected is given in Table 2.

Four of 16 mammalian species examined were infected, and in every instance the first infection was found in the first to the eighth animal collected. The gray fox and the cotton mouse are the only ones of the 12 species not found infected in which more than six specimens were examined. The only adult worm recovered from the infected mammals was a mature female found between the scapula and thoracic wall of a rabbit which did not have microfilariae in the lungs. Larvae were common in muscle and other tissues of the two species of squirrels and in the rabbit. This could indicate the absence of strict nocturnal periodicity, but evidence from so few animals is inconclusive.

The complete absence of infection among 137 specimens of 35 species of cold-blooded animals suggests that if the group is parasitized by filarial worms, the rate is extremely low. However, data from so few animals cannot be conclusive for any single species.

Birds represent the majority of animals examined since they were the easiest

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TABLE 1.—*Species infected with filarial worms*[1]

Scientific name	Common name	Number examined	Number infected
Aves			
Corvidae:			
Corvus brachyrhynchos	Eastern crow	61	38
Cyanocitta cristata	Blue jay	191	100[2]
Laniidae:			
Lanius ludovicianus	Loggerhead shrike	21	5
Picidae:			
Centurus carolinus	Red-bellied woodpecker	62	2[3]
Melanerpes erythrocephalus	Red-headed woodpecker	49	2[4]
Dendrocopos pubescens	Downy woodpecker	30	2[5]
D. villosus	Hairy woodpecker	6	1
Cathartidae:			
Cathartes aura	Turkey vulture	3	2[13]
Coragyps atratus	Black vulture	4	4[13]
Mimidae:			
Mimus polyglottos	Mockingbird	46	6[6]
Toxostoma rufum	Brown thrasher	27	1[14]
Hirundinidae:			
Progne subis	Purple martin	1	1
Fringillidae:			
Pipilo erythrophthalmus (probably 3 subspecies)	Towhee	27	8[15]
Zonotrichia albicollis	White-throated sparrow	15	1[6]
Richmondena cardinalis	Cardinal	46	12[6]
Troglodytidae:			
Thryothorus ludovicianus	Carolina wren	19	1[6]
Turdidae:			
Sialia sialis	Bluebird	29	7[6]
Sylviidae:			
Polioptila caerulea	Blue-gray gnatcatcher	10	3
Vireonidae:			
Vireo griseus	White-eyed vireo	7	4[7, 6]
V. olivaceus	Red-eyed vireo	14	3[8, 6]
V. flavifrons	Yellow-throated vireo	12	3
Parulidae:			
Protonotaria citrea	Prothonotary warbler	3	1
Parula americana	Parula warbler	20	3[7]
Caprimulgidae:			
Chordeiles minor	Nighthawk	5	1[12]
Thraupidae:			
Piranga rubra	Summer tanager	20	5
Paridae:			
Parus bicolor	Tufted titmouse	37	2
Sittidae:			
Sitta pusilla	Brown-headed nuthatch	8	1
Tyrannidae:			
Tyrannus tyrannus	Kingbird	25	5[9]
Myriarchis crinitus	Crested flycatcher	33	1
Ardeidae:			
Florida caerulea	Little blue heron	5	1[10]
Columbidae:			
Columbigallinae passerina	Ground dove	10	1
Icteridae:			
Sturnella magna argutula	Meadowlark	19	2[4]
Agelaius phoeniceus	Red-winged blackbird	5	1
Cuculidae:			
Coccyzus americanus	Yellow-billed cuckoo	10	4
	Total	880	234
Mammalia			
Leporidae:			
Sylvilagus floridanus mallurus	Cottontail rabbit	8	2[11]
Sciuridae:			
Sciurus niger	Fox squirrel	5	1
S. carolinensis	Gray squirrel	30	4
Mustelidae:			
Mephitis mephitis elongata	Striped skunk	1	1
	Total	44	8

[1] Infections are with microfilariae, except when otherwise noted.
[2] Two species of microfilariae, eggs, and two or three species of adult worms; one bird with eggs, microfilariae, and adults.
[3] One specimen with adults and eggs, one with adults and microfilariae.
[4] Adults only.
[5] Adults and embryonated eggs in one specimen, adults only in the other.
[6] Two species of microfilariae.
[7] Adult and microfilariae.
[8] Adult, two species of microfilariae.
[9] Five species of microfilariae.
[10] One microfilaria only.
[11] One with an adult, one with microfilariae.
[12] Adults and embryonated eggs.
[13] The same species in both members of the family.
[14] The same species as one of those in the mockingbird.
[15] Three species of microfilariae.

TABLE 2.—*Species not infected with filarial worms*

Scientific name	Common name	Number examined
Amphibia		
Plethodontidae:		
Plethodon sp.		1
Eurycea bislineata cirrigera	Southern two-lined salamander	2
Pseudotriton ruber vioscai	Viosca's red salamander	2
Hylidae:		
Acris gryllus	Cricket frog	3
Hyla gratiosa	Georgia tree frog	1
Pseudacris ornata	Ornate tree frog	1
Ranidae:		
Rana pipiens sphenocephala	Leopard frog	5
Brevicipitidae:		
Microhyla carolinensis	Narrow-mouthed toad	1
Bufonidae:		
Bufo terrestris	Southern toad	3
	Total	19
Reptilia		
Emydidae:		
Pseudemys scripta	Yellow-bellied turtle	9
P. floridana	Coastal plain turtle	3
Terrapene carolina triunguis	Three-toed box turtle	12
T. c. major	Gulf Coast box turtle	1[1]
Deirochelys reticularia	Chicken turtle	4
Kinosternidae:		
Kinosternon subrubrum	Mud turtle	5
Testudinidae:		
Gopherus polyphemus	Gopher turtle	3
Chelydridae:		
Chelydra serpentina	Snapping turtle	1
Iguanidae:		
Anolis carolinensis	Chameleon	6
Sceloporus undulatus	Fence lizard	16
Scincidae:		
Leiolopisma laterale	Brown skink	6
Eumeces laticeps	Greater five-lined skink	9
Teiidae:		
Cnemidophorus sexlineatus	Six-lined race runner	17
Colubridae:		
Masticophis flagellum	Coachwhip snake	4
Lampropeltis getulus getulus	King snake	2
Elaphe obsoleta obsoleta	Pilot blacksnake	1
E. o. confinis	Gray rat-snake	1
Coluber constrictor	Blacksnake	2
Pituophis melanoleucus mugitus	Southern pine snake	1
Diadophis punctatus punctatus	Ring-necked snake	1
Natrix erythrogaster flavigaster	Red-bellied watersnake	1
Opheodrys aestivus	Keeled green snake	1
Heterodon simus	Hog-nosed snake	2
Crotalidae:		
Crotalus adamanteus	Diamond-backed rattlesnake	2
Agkistrodon piscivorus	Water moccasin	3
Crocodilidae:		
Alligator mississippiensis	Alligator	5
	Total	118
Aves		
Picidae:		
Colaptes auratus	Flicker	5
Hylatomus pileatus	Pileated woodpecker	2
Fringillidae:		
Zonotrichia leucophrys	White-crowned sparrow	1
Aimophila aestivalis	Pine woods sparrow	5
Parulidae:		
Dendroica pinus	Pine warbler	17
D. dominica	Yellow-throated warbler	1
Mniotilta varia	Black and white warbler	5
Seiurus motacilla	Water thrush	1
Vermivora perigrina	Tennessee warbler	1
Caprimulgidae:		
Caprimulgus carolinensis	Chuck will's widow	2
Paridae:		
Parus carolinensis	Chickadee	25
Sittidae:		
Sitta carolinensis	White-breasted nuthatch	3
Tyrannidae:		
Empidonax virescens	Acadian flycatcher	6
Contopus virens	Wood peewee	1

TABLE 2.—*Continued.*

Scientific name	Common name	Number examined
Ardeidae :		
Casmerodius albus egretta	American egret	1
Nyctanassa violacea	Yellow-crowned night heron	1
Butorides virescens	Green heron	1
Icteridae :		
Dolichonyx oryzivorus	Bobolink	1
Icterus spurius	Orchard oriole	3
Quiscalus quiscula	Purple grackle	1
Accipitrinae :		
Accipiter cooperii	Cooper's hawk	1
Buteoninae :		
Buteo lineatus	Red-shouldered hawk	5
B. jamaicensis	Red-tailed hawk	1
Falconinae :		
Falco sparverius	Sparrow hawk	14
Ploceidae :		
Passer domesticus	English sparrow	10
Sturnidae :		
Sturnus vulgaris	Starling	1
Charadriidae :		
Charadrius vociferus	Killdeer	1
Phasianidae :		
Colinus virginianus	Quail	2
Columbidae :		
Zenaidura macroura	Mourning dove	2
Tytonidae :		
Bubo virginianus	Great horned owl	1
Threskiornithidae :		
Guara alba	White ibis	2
Alcedinidae :		
Megaceryle alcyon	Belted kingfisher	1
Unidentified fledglings		2
	Total	126
Mammalia		
Felidae :		
Lynx rufus	Florida bobcat	2
Canidae :		
Urocyon cinereoargenteus	Gray fox	11
Vulpes fulva	Red fox	1
Procyonidae :		
Procyon lotor	Raccoon	3
Didelphiidae :		
Didelphis virginiana	Opossum	5
Leporidae :		
Sylvilagus palustris	Swamp rabbit	1
Talpidae :		
Scalopus aquaticus	Common mole	1
Muridae :		
Rattus norvegicus	Norway rat	6
Mus musculus	House mouse	3
Cricetidae :		
Peromyscus gossypinus	Cotton mouse	15
Unidentified bat		1
Unidentified shrew		1
	Total	50

[1] Collected in Leon County, Florida.

of the vertebrates to collect in large numbers and showed the highest rates of infection.

Fifty-one per cent of the 66 species of birds collected were infected with microfilariae, adult worms, or both; 86 per cent of the 28 species in which 10 or more specimens were examined were infected; 25 per cent of the species in which fewer than 10 specimens were examined were infected; and 24 per cent of all birds examined were hosts of filarial worms. With one exception, the first parasitized individual was found among the first 14 birds of a species collected; the exception was the red-bellied woodpecker, in which the thirty-eighth bird harbored an adult worm and filarioid eggs, and the fifty-fourth harbored both adult worms and microfilariae.

The Picidae and Paridae show the lowest rates of infection of all the infected avian species. One hundred and fifty-four woodpeckers of six species were examined, and only seven of them were infected. Microfilariae were found in two of the seven; filarial eggs were found in two others; and adult worms were found in six of the seven. The 25 chickadees collected were not infected, and only the first and third of 37 tufted titmice examined had microfilariae. Conclusive data on rates of infection in most infected species are not available, because usually few birds of a species were collected after one or two infections were found.

Immature specimens of *Thryothorus ludovicianus, Corvus brachyrhynchos, Pipilo erythrophthalmus, Vireo olivaceus, Piranga rubra, Lanius ludovicianus, Cyanocitta cristata, Richmondena cardinalis, Polioptila caerulea, Agelaius phoeniceus,* and *Tyrannus tyrannus* were found infected with microfilariae during the nesting season or shortly thereafter. The relative age of the birds was estimated by unmistakable characteristics, such as juvenile plumage, soft mouth corners, unwariness, and poor flying ability. No doubtful specimen was recorded as juvenile; more birds of these and perhaps of other species would have been recognized as juveniles if more precise methods of age determination had been applied. The youngest infected bird probably was a blue jay estimated to have left the nest not more than two weeks before collection.

Most of the birds collected were nonmigratory, so the question of the origin of the infections is a minor one. Infected immature specimens of three of the migratory species, *T. tyrannus, P. rubra,* and *V. olivaceus,* were recorded. None of the juvenile crested flycatchers collected during the summer were infected; the only infection in this species was in a mature specimen shot within a week after the birds appeared in the survey area.

The number of species of microfilariae, their degree of host specificity, and their distribution among the avian species, cannot be determined until morphological studies in progress are completed. It is certain in a number of instances that one species of worm is the usual filarial parasite in an avian species. In other instances, two or more species of filarial worms sometimes are found in an individual bird or in one species.

All the infected crows examined harbored a single species of microfilaria, and the first 106 infected blue jays harbored another species. Three of these jays also were infected with a visibly different type of microfilaria. In addition, at least two species of adult worms have been recovered from blue jays. Five infected kingbirds contained five kinds of microfilariae; if the species are identified by the letters A to E, two birds harbored type A, one had types B and C, one had types C and D, and one bird had type E. It is apparent that none of these can be designated as the usual species to be found in kingbirds. A Carolina wren, a white-throated sparrow, and two red-eyed vireos each were infected with two species of microfilariae. Towhees, cardinals, bluebirds, white-eyed vireos, and mockingbirds were found to harbor two or three kinds of microfilariae, but without double infections in single birds. Red-bellied woodpeckers contained two kinds of larvae, one free and the other type in embryonated eggs. Conversely, the microfilariae from both species of Cathartidae are specifically identical, as are the blunt-tailed larvae found in both species of Mimidae, and the microfilariae from the two species of Picidae.

Large numbers of blue jays were collected because that species was selected for an epidemiological study of the filarial infection. Data from the first 200 birds, collected from March to December, show a summer-time drop in the infections to a minimum of only one-third of the spring and fall rates.

Ectoparasites were collected from all the animals examined, to reveal any possible correlation between these and filarial infections. Mallophaga were the only external parasites present frequently enough to be considered as possible vectors, and none of the data suggested that they are connected in any way with the filaria life cycles.

Both the adult worms and the microfilariae were remarkably localized in the birds. Adult worms were found in 22 birds, and in 19 of them were in coelomic spaces, mesenteries, or associated blood vessels. The worms were in the abdominal mesenteries of the downy woodpecker, in the blood vessels of the abdominal wall and mesenteries in the meadowlark, in the pleural and abdominal cavities of the red-bellied woodpecker, and either free or in connective tissue in the thoracic region of the other birds. Migration after death could account for the presence of the worms in the cavities, but three filariae were found in the pleural region of a blue jay immediately after it was killed. The worms from the nighthawk were beneath the dorsal buccal mucosa. The location of the adult worms from the two remaining birds is unknown.

The majority of the microfilariae invariably were found in the lungs. Often a few could be found in the heart blood, but usually none were in the spleen, liver, pancreas, kidney, brain, or skeletal muscle. The concentration of the microfilariae in the lungs of birds dying during daylight hours, and their absence from all other organs, strongly suggests a strict nocturnal periodicity of all the avian filaria species collected. Filarial worms in the crow are known to be nocturnally periodic (Boughton, Byrd, and Lund, 1938), and show such periodicity in this laboratory. No microfilariae have been found in mosquitoes fed on infected blue jays in this laboratory during the daytime, but can be found with this technique within an hour after sunset. The nighthawk was killed and examined at dusk, and contained numerous embryonated eggs but no microfilariae.

Only one microfilaria was found in ten smears of various tissues from the little blue heron, and that one was remarkable for its large size. It was easily visible with 9× magnification in the saline smear, swimming among the blood cells. It measured about one mm. in length after staining, had microfilarial structures, and was slightly differentiated at the anterior end.

The major problem which developed from the survey is the difficulty in correlating adults, microfilariae, and eggs in individual birds or in single species. Four factors are responsible.

1. Failure to recover adult worms, particularly types not inhabiting the coelom, from infected birds. It is assumed that most hosts with microfilariae or filarioid eggs in the lungs should contain adult worms. Forty-six birds of 13 species were examined for adults by the technique described. All of these birds were heavily infected with microfilariae, but adults were discovered in only two of them. The filariae recovered in this way were a single minute male from a red-eyed vireo, which harbored two kinds of microfilariae, and two small and thread-like mature females from a blue jay. In contrast, worms readily visible during routine dissec-

tions without special search were found in 20 other birds. Stout, opaque worms from two to 17 cm. long were found in the coelomic cavities of eight blue jays containing microfilariae and one containing microfilariae and eggs; in two red-bellied woodpeckers, one of which had microfilariae and the other embryonated eggs; in two downy woodpeckers, one with embryonated eggs in the tissues; in two meadowlarks and one parula warbler with neither eggs nor microfilariae; and in one white-eyed vireo with microfilariae, in addition to the worms and embryonated eggs from the night hawk. None of these birds were examined for smaller, tissue-inhabiting adults.

2. Failure to find microfilariae or eggs in birds containing adult worms. Neither eggs nor larvae were detected in six birds listed above in which one or more adults were found.

3. The possibility of more than one filarial species inhabiting a single avian species. Multiple infections (exclusive of adults) were, in fact, found in 9 different birds of 5 species. Six additional avian species were infected with more than one filarial species without multiple infections being discovered in single hosts.

4. The presumed presence both of oviparous and ovoviviparous species, and ignorance of the fate of filarioid eggs in the tissues. Most infected birds contained only microfilariae. Eggs and microfilariae were found together in but one bird, a blue jay. No microfilariae could be detected in either a downy woodpecker or a nighthawk containing adult worms and numerous embryonated eggs, nor in a red-bellied woodpecker with adults and a very few embryonated eggs.

The presence or absence of adult worms, microfilariae, and eggs have occurred in various combinations. One or more species of filariae have been found in deep tissues or coelomic spaces of each of nine species of birds. These worms have been associated with either a complete lack of eggs and larvae, one or two kinds of microfilariae, embryonated filarioid eggs, or both eggs and larvae. Conversely, 215 birds lacked adult worms in the coelom, but contained microfilariae or eggs. Tissue-inhabiting adults were recovered from only three of these specimens. Of the remaining 212, 44 were examined by the saline technique described, but did not yield adults. Microfilariae and the larger adults inhabiting the coelom probably rarely were overlooked, due in the first instance to the movement and in the second to the large size and relative freedom from surrounding tissues. The smaller and more thread-like filariae could have been overlooked quite frequently in dissections, and it is possible that the warm saline treatment was only slightly successful in attracting them from the tissues.

DISCUSSION

One objective of the survey was to find the extent of filarial infections in the local vertebrate population. Field identification manuals list about 40 mammalian, 96 avian, 60 reptilian and 42 amphibian species in southwestern Georgia; some of these are rare, or do not occur in the limited area surveyed. A total of 117 species was collected, but only 34 are represented by as many as 10 specimens. It is apparent that a theoretically complete and adequate survey was not accomplished. The majority of the most common and easily collected vertebrate species were examined, however, some in considerable numbers.

The data show that in the populations examined some avian species are com-

monly infected with filarial nematodes, many more are occasionally infected, most of the warm-blooded species should be found infected at least occasionally if enough specimens are examined, and that infections are rare or absent among reptiles and amphibia.

Avian species may be grouped into three categories with respect to rate of infection. Some show rates of 20 per cent to 50 per cent or higher, so the vectors must be efficient or common; the Corvidae, towhee, cardinal, shrike, and probably the Vireonidae, Cathartidae, kingbird, summer tanager, bluebird, cuckoo, mockingbird, and blue-gray gnatcatcher belong in this group. Many species have such low infection rates that the infections either are accidental, or the vectors are inefficient or scarce; the white-throated sparrow, the three more numerous species of Picidae, the hairy woodpecker, tufted titmouse, ground dove, brown thrasher, parula warbler, meadowlark, brown-headed nuthatch, and Carolina wren probably belong in this group. It is possible that some species lack filarial infections, or the vectors are not present in this area; the chickadee, pine warbler, and sparrow hawk may belong in this group, unless further collections show them to be in the second group. The failure to find any infected juvenile flycatchers and the finding of only one infected adult early in the summer could mean that these migratory birds have a low rate of infection, and do not become infected in the survey area. Species in which only one or a few birds have been examined cannot be fitted into one of these categories, but the proportion of infected individuals in some of these species suggests that they belong in the first group.

The collection of infected juvenile birds of 11 species shows that the vectors are active during the nesting season, and that the infection can be and frequently is contracted in the nest. Nesting in the area starts during April, and juvenile birds were shot from the middle of May to late August. The maximum time necessary for development of the worms to maturity in the birds therefore should be no more than three to four months. The minimum time for this development in at least one species is five weeks or less, if it is assumed that blue jays remain in the nest for three weeks after hatching, and that the presumed youngest infected bird had been out of the nest for about two weeks, as estimated. These facts make it likely that life cycle experiments using these birds as hosts would be conservative of time, and that the vector is an arthropod which will attack the birds in the nest.

The degree of host specificity among the filarial worms collected in this survey has not yet been revealed. Indications are that the avian species with high infection rates are characterized by a particular kind of filarial infection, but there may be occasional cross-infections between host species. It also is possible that in cases of multiple infection, such as that shown by the kingbirds, several species of filarial worms are normal parasites of one bird species, so that the principle of host specificity is not invalidated. It may be, too, that a few filarial species are capable of infecting a wide range of hosts, thus accounting for multiple infections in avian hosts which normally are parasitized by only one filarial species. Completion of the morphological studies should give the answers to some of these problems.

The location of the microfilariae in the lungs supports the hypothesis of Hawking and Thurston (1951) that the lung is the normal and primary seat of the microfilarial infection in nocturnally periodic species, and that even during the time of maximum peripheral concentration most of the total number still are in the lungs.

Certainly the vast majority of the larvae are found in the lungs of the infected birds, and it should be possible to discover what proportion of them leave that organ at night for the peripheral vessels.

An explanation for the scarcity of microfilariae in the little blue heron, and the large size of the one found, could be that the larva was a survivor of a very old infection, and that it had grown and differentiated beyond the normal appearance.

It probably will not be feasible to correlate adult and larval worms in the birds until morphological and life cycle studies have been completed. A plausible hypothesis to explain the facts as now known is that birds are host to two major groups of filarial worms. One group would contain relatively long, stout, opaque, easily seen adults living in the body cavities. The other group would consist of small, transparent, thread-like adults living in deeper tissues. The first group would be generally oviparous, and the second ovoviviparous. Finally, the short developmental period shown by the worms in juvenile birds could indicate a short adult life, so that filariae sometimes would be absent when some microfilariae survived in the lungs. This latter supposition is supported by numerous infections in which microfilariae are very scarce.

Most of the findings can be explained by this hypothesis. The 214 birds with microfilariae but apparently no adults in the body cavity were parasitized by the tissue-inhabiting type of worm. Such worms were recovered from two of them. In the remaining 212 specimens, the adults either were not visible in routine dissections, had died and degenerated, or were overlooked in 44 of the 46 heavily infected birds in which a special search was made. Alternatively, some of the 212 birds had been infected with coelom-dwelling adults which had died and degenerated prior to the examination. This situation is unlikely in the 46 heavily infected birds, because the large numbers of microfilariae should indicate the presence of active female worms. The 11 birds harboring microfilariae and coelom-dwelling adults should have contained eggs and tissue-dwelling adults as well. The supposition of a double infection is supported by the fact that one blue jay indeed did have both eggs and microfilariae, as well as coelom-dwelling adults. Alternatively, these birds were singly infected with the coelom-dwelling adults, and, except in the one blue jay, all of the eggs had hatched by the time of the examination. This may be considered unlikely unless a very strict cycle both of oviposition and of hatching prevailed. The nighthawk, and the two woodpeckers with coelom-dwelling adults and eggs in the lungs, were singly infected with an oviparous species; the complete absence of microfilariae also can best be explained by a strict cycle of oviposition and hatching. The blue jay and red-eyed vireo with tissue-dwelling adults and microfilariae were singly infected with an ovoviviparous species. Eggs should have been found in the lungs of the six birds harboring filariae in the coelom but with no microfilariae or eggs in the tissues. The absence of eggs is not presently explicable except as an aberration.

Verification or modification of this hypothesis, or development of an alternative one, depends on accurate study of larger numbers of infected birds, and on the working of filarial life cycles under controlled conditions.

The considerable number of multiple infections with microfilariae, combined with the scarcity of information on the occurrence of the adult worms, shows that the same specific name cannot safely be applied both to larval and adult worms

taken from one bird or an avian species, except under special conditions. The best circumstance, not routinely obtainable in practice, is to do the life cycle under controlled conditions. Next best is to accumulate such a mass of consistent data from field collections that the likelihood of the larvae and adults concerned being of the same species is substantially supported.

SUMMARY

Of the 1,237 vertebrates representing 117 species examined for filarial worms, 242 animals of 38 species were found infected. The majority of the infections were with microfilariae, but filarioid eggs and adult worms occasionally were found. Birds constituted 1,006 of the animals examined, and 234 of them were infected; among that group, 51 per cent of the species collected, and 86 per cent of the species represented by 10 or more specimens, were infected. Four of the 16 mammalian species and eight of 94 individuals examined were infected. None of 137 reptiles and amphibia of 35 species were found infected.

Juvenile specimens of 11 species of birds harbored microfilariae. Three of these species are migratory, but breed in the survey area. Adult worms were found in 22 birds, but not in 44 others which were specifically examined for them. Nine individuals and 11 species were found with multiple infections.

Most of the adult worms were found associated with the coelom, but the difficulty of finding delicate tissue-dwelling filariae may account for the apparent scarcity of that type. In birds, the microfilariae invariably were found primarily in the lungs, secondarily in the heart, and almost never in other tissues. Nocturnal periodicity is indicated by these facts and by experimental evidence in the blue jay and crow.

Larval and adult worms could not be correlated with certainty because of the lack of adult worms, or the difficulty in finding them; the multiple infections; and the probable presence of oviparous and ovoviviparous species. It is suggested that two groups of filarial worms parasitize birds, one group consisting of stout, opaque, easily seen, oviparous worms dwelling in the coelomic spaces, the other of small, thread-like, transparent, ovoviviparous worms living in deeper tissues.

Several common avian species regularly have an infection rate of 20 per cent or more; material for life cycle work and morphological studies can best come from this group; the infections in birds can be contracted in the nest, and often are; some worms develop to maturity in the host in as few as six weeks, and others in no more than four months; host specificity among the avian filarial worms will be found, but multiple infections of individuals and species, and identical infections in closely related bird species, will occur.

It is necessary to make extensive morphological studies on microfilariae and adults, and to elucidate life cycles of avian filariae before eggs, larvae, and adults can be correlated.

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STUDIES ON MARINE DIGENETIC TREMATODES OF PUERTO RICO: THE FAMILY BIVESICULIDAE, ITS BIOLOGY AND AFFINITIES[1]

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INTRODUCTION

A survey of larval marine trematodes in Puerto Rico disclosed a type of cystocercous cercaria, of which six species were eventually found to develop in snails of the genus *Cerithium*. Because these larvae presented several unusual features, the writer undertook a further investigation of them. In so doing, it became evident that their adults are members of the family Bivesiculidae, a peculiar group of intestinal parasites occurring in marine fishes.

The first representative of the family was described by Yamaguti (1934) who erected the genus *Bivesicula* for a single species, *B. claviformis*. For the genus, he proposed the subfamily Bivesiculinae which was assigned to the family Monorchiidae. Recently Nagaty (1948) reported *B. claviformis* from *Serranus fasciatus* in the Red Sea. In 1938, Yamaguti described 2 additional species of *Bivesicula*, *B. synodi* and *B. epinepheli*, and erected the genus *Bivesiculoides* for a related species, *Bivesiculoides atherinae*. The next year Yamaguti (1939) raised the subfamily Bivesiculinae to the rank of family, Bivesiculidae. Manter (1947) described from Tortugas, Florida, *Bivesicula hepsetiae*, the only representative of the family known from the Atlantic and Crowcroft (1947) indicated still further extent of the group in describing *Bivesicula australia* from Tasmania.

Although Yamaguti originally referred these trematodes to the Monorchiidae, an affinity with that family was not suggested later when he separated from it the Bivesiculidae as an independent family. However, Manter (1947) was of the opinion that the bivesiculids are closely related to the monorchiids. This view is not in accord with the writer's observations on larval stages as reported in a recent abstract (Le Zotte, 1952). Life history studies, which have been so fruitful in elucidating affinities of other groups, have been lacking so far as the Bivesiculidae is concerned. However, what is obviously the cercaria of a member of this group was included merely as a photomicrograph in a recent paper by Hutton (1952).

MATERIALS AND METHODS

The usual methods of studying cercariae were used. Care was necessary, however, that data of a descriptive nature be obtained from fully developed larvae because of a tendency to emerge before the body is retracted into the tail or even full size is attained. This was especially true after infected snails were kept in the laboratory for a period of time, because often redia as well as incompletely developed

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[1] Based on a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Purdue University. This study was made during 1951–52 when the writer had the opportunity to work under the direction of Prof. R. M. Cable (Fellow, John Simon Guggenheim Foundation), who was then engaged in investigating the marine digenetic trematodes of Puerto Rico. The writer wishes to acknowledge also the assistance of Profs. N. T. Mattox, Luis R. Rivas, and Mr. Donald Erdman in the collection and identification of hosts.

cercariae escaped from them. Prematurely emerging cercariae were weak and did not swim in the normal manner. These larvae may differ to such an extent from the fully developed ones as to make identification difficult. For these reasons freshly emerged, active larvae with bodies retracted into the tail were used for measurements after being killed in near-boiling sea water and transferred to a slide with an excess of water to avoid cover glass pressure. By this method specimens were remarkably uniform in size and sources of error were minimized.

Infected snails were cracked to obtain developmental stages. Both larvae and adults were fixed in corrosive sublimate-acetic acid and stained with either Harris' hematoxylin or Semichon's carmine.

Cercariae described in this paper are given the same designations under which they are to be included by Prof. R. M. Cable in a projected monograph of the marine larval trematodes of Puerto Rico.

LIFE CYCLE OF THE BIVESICULIDAE

The evidence at hand concerning the bivesiculid life cycle is not beyond question; technical difficulties prevented experimental studies of a conclusive nature. In the first place, species in this family are evidently highly specific in respect to their definitive hosts. Although hundreds of fishes representing dozens of species were examined and immature bivesiculids were found repeatedly, only *Bivesicula hepsetiae* was obtained sexually mature in natural infections. This species was found in the mature state only in *Atherinomorus stipes* (= *Hepsetia stipes*), the same host from which Manter (1947) reported *B. hepsetiae* at Tortugas, Florida.

Although several species of fish were exposed to cercariae, adult worms were not recovered from any of them. This was not surprising because the chances of finding a suitable definitive host by this method were remote indeed. Difficulty was experienced in obtaining *A. stipes* alive and maintaining it in the laboratory for experiments with the cercaria believed to be the larva of *Bivesicula hepsetiae,* which occurs naturally in that host.

There is good evidence that the life cycle in the BIVESICULIDAE, as in the AZYGIIDAE, does not include a second intermediate host. Instead, it seems that the definitive host becomes infected by ingesting the cercaria. The larva is large and an active swimmer; because of these characteristics and its positive reaction to light, the cercaria would attract the attention of the small fishes which are predominantly hosts of the described species of the family. Although cephalic glands are present, there is no indication of cystogenous glands and their absence indicates the lack of an encysted metacercarial stage at least. Finally, the immature worms found repeatedly in the intestine of fishes, were no different in size and appearance from the cercarial body. These all are facts suggesting that the cercariae develop directly to maturity in the intestine of suitable fishes which ingest them.

On an ecological basis, it is believed that the species described below as *Cercaria caribbea XLIII* is the larva of *Bivesicula hepsetiae,* but this cercaria is not so designated because experimental proof is lacking.

Whether or not the life cycle in the BIVESICULIDAE requires a second intermediate host, there can be no question that the cercariae described below are the larvae of members of that family. The unique situation in respect to the digestive and excretory systems and complete agreement between the cercarial stage and the adult leave no room for doubt.

MORPHOLOGY OF THE BIVESICULIDAE

(All measurements in millimeters)

Bivesicula hepsetia Manter, 1947

Adult (Figs. 22–25)

Fifty-five of 109 hardhead silversides, *Atherinomorus stipes,* were found to be infected with *B. hepsetiae.* This incidence is much higher than reported by Manter (1947) who stated, however, that this trematode would be easily overlooked because of its small size. The writer found one to 14 specimens with an average of 2.54 per infected fish. His specimens are in general agreement with the original description which may be extended and supplemented, especially in regard to the reproductive and excretory systems.

A *receptaculum seminis uterinum* is present as described for other species of *Bivesicula.* The structure interpreted by Manter as a seminal receptacle was studied at length in living specimens and found to be a thick-walled modification of the oviduct which may contain spermatozoa and function as a fertilization chamber; it has the form of a small blind sac whose structure and position with reference to Laurer's canal distinguish it from the usual type of seminal receptacle. Other details supplementing Manter's description of the reproductive system are shown in Fig. 24.

A single excretory pore has been described for adult bivesiculids. In *B. hepsetiae* at least and probably all other species to judge from observations on several cercariae described below, there actually are two distinct vesicles each with its own sphincter and pore. Not until after decaudation is there any suggestion of the common passage which has been described as a single excretory pore but actually is a secondary invagination at the posterior end of the body (Fig. 23).

The pharynx-like appearance of what has been interpreted as an oral sucker is mentioned in several descriptions and a rudimentary pharynx posterior to this structure is described in some instances. The esophagus in *B. hepsetiae* is muscular but no more so than in several trematodes possessing a distinct pharynx as well as an oral sucker. For this reason, what has been interpreted as a pharynx may actually be a muscular esophagus and the so-called oral sucker a true pharynx. Sections of both adult and cercariae within the redia (Figs. 26–28) show no appreciable histological difference between the redial pharynx and the structure in the cercaria or adult. Radial and circular muscles are the same and there is a similar arrangement of nuclei, their being concentrated posteriorly.

Egg and Miracidium (Fig. 25)

In *B. hepsetiae* the eggs are large (0.075–0.080 by 0.045–0.050 in living material), operculate, and few in number; the shell is smooth and thin. When eggs were teased from the worm and placed in standing sea water at room temperature, development occurred normally in some of them. The eyespot of the miracidium appeared on the 6th to 7th day as a pair of well-separated pigment cups which with further development moved together to form a united structure. About two weeks are required for the miracidium to become fully developed. The larva has distinct epidermal plates bearing long cilia and resembles the miracidia of *Clinostomum* and strigeoids more than those of azygiids. The structure of the miracidium indicates that it normally hatches and penetrates the molluscan host. However, all attempts

to induce hatching were unsuccessful. After reaching full development and exhibiting much motility, the miracidia died and disintegrated. Thus what is known of miracidial structure was observed through the egg shell. No flame cells could be observed.

The Redia (Figs. 20, 21, 27, 29, 30)

All species of bivesiculid cercariae described below develop in rediae in the branchial region of the snail host. The redia has a cleft posterior extremity and transverse muscle bands which are prominent in the contracted state. Under the lower powers of a dissecting microscope, young redia (Fig. 20) are easily confused with cercarial embryos when the infected snail is cracked.

A golden-brown pigment is discernible throughout the entire body of the redia. The gut is usually filled with a darker brown pigment. The older redia of *Cercaria caribbea XLV* measures 0.441 to 0.688 in length and 0.125 to 0.213 in width. The pharynx is almost spherical, measuring 0.025 to 0.033 in diameter. The gut is short, 0.092 to 0.134 long. Longitudinal sections show what probably is a birth pore slightly posterior to the pharynx. A discrete mass of germinal cells is present; in all rediae observed it was attached to the median wall of one of the posterior lobes and close to their confluence so that the mass is to one side of the mid-line of the redia (Fig. 30).

The excretory pattern was determined for the redia of *Cercaria caribbea XLV* (Fig. 21). The pores are at the tips of the posterior lobes and from each pore an excretory canal extends anteriorly, receiving an anterior and a posterior collecting tubule. The anterior collecting tubule is joined by capillaries of two groups of flame cells of which four were observed in the anterior group and three in the second. The posterior collecting tubule receives capillaries from three groups of flame cells, the two more anterior of which also are in three's, while the posterior group contains four flame cells. In groups containing four flame cells, the common tubule divides and both of the resulting tubules divide again to form four capillaries. In groups containing three flame cells, the common tubule divides but only one of the resulting tubules divides again to form capillaries, the other serving a single flame cell. The excretory formula for this redia accordingly is $2[(4+3)+(3+3+4)] = 34$ flame cells, a pattern which is very similar to that of the cercaria, as described below.

Cercariae

Bivesiculid cercariae evidently escape from the redia and complete their development in the tissues of the snail. All the six species here reported have many features in common. Thus, repetition may be avoided by first giving a:

General diagnosis: Furcocystocercous cercariae developing in rediae in the branchial region of marine prosobranch snails. Photopositive, swimming tail first and resting intermittently. Tail often characteristically pigmented, with vacuolated parenchyma, and divided into a vesicle into which the body is withdrawn, a stem sharply set off from the vesicle, and a pair of furcae; tail stem and furcae laterally compressed. The vesicle has powerful circular muscles and is concave posteriorly to receive the base of the tail stem. In fully developed cercariae, the body is enclosed in a spherical muscular capsule which has radial fibers and does not occupy

all of the caudal vesicle. When containing the body, the capsule is distended and thin but is much smaller and thick-walled when empty (Figs. 3, 14). The body when free is rounded anteriorly and attenuated posteriorly. It is covered with minute spines and body pigment is limited to a pair of conspicuous eyespots. Oral and ventral suckers are lacking, there being a well-developed pharynx, heretofore interpreted as an oral sucker, and anterior to it a short but distinct vestibule. Esophagus muscular; intestine triclad with long ceca extending to near the posterior end of the body. Numerous cephalic glands are situated near the intestinal bifurcation and have ducts opening into the vestibule (Fig. 9). Cystogenous glands lacking.

The genital primordium is median, intercecal, in the posterior fourth of the body and sometimes shows indication of dissociation to form the gonads and their ducts.

The right and left halves of the excretory system remain separate throughout development. The pair of embryonic excretory tubules extend through the tail to diverge and open at the tips of the furcae. Consequently the body contains two separate thin-walled excretory vesicles each with its own sphincter (Figs. 8, 31). The excretory vesicles usually are filled with spherical concretions. From the anterior end of each vesicle, a ciliated recurrent tubule extends posteriorly, passing first medially and then dorsal to the vesicle to receive at about mid-body level an anterior and a posterior collecting tubule. The anterior tubule receives capillaries from two groups of flame cells while the posterior is joined by three flame cell groups, the last of which is in the tail. Each flame cell group has dichotomous branching of the capillaries so that the caudal vesicle receives one-half of the posterior-most flame cell group and the proximal region of the tail stem the other half. Empirically, the excretory formula is $2[(n+n)+(n+n+n)] = 10$ n flame cells.

The embryology of the excretory system (Figs. 15–19) was traced to the extent of observing the major changes during development. In the young embryo each primary tubule extends anteriorly and expands as a small ciliated chamber from which a tubule loops posteriorly and divides into capillaries with a pair of flame cells. Then as the embryo elongates the first suggestion of the anterior-posterior asymmetry of the system appears when a flame cell is added to the posterior tubule on each side. By the time primordia of the eyespots and the tail region of the embryo are evident, each of the ultimate five groups of flame cells is represented by a single flame cell and with further development these cells become paired so that there are 10 flame cells on each side, the posterior pair being in the developing tail. Finally, the number is doubled to give the complete pattern of the emerging cercaria.

Cercaria caribbea XLIII

(Fig. 1)

Specific diagnosis; Species of moderate size with the general characters of the group. Rests occasionally with the body downward and furcae spread. The anterior end of the caudal vesicle with a yellowish pigment which fades posteriorly. Just posterior to the body capsule there is an area which appears lighter, is devoid of pigment and may be a cavity. Tail stem and furcae without obvious pigment. Eyespots 0.010 long and 0.014 wide. Pharynx 0.028 long and 0.034 wide. Capsule with body in it 0.115 to 0.145 in diameter. Caudal vesicle 0.275 to 0.287 long and 0.281 to 0.308 wide; tail stem 0.411–0.425 long measured on lateral surface from the base of furcae to attachment to vesicle and 0.056 in maximum width. Furcae 0.165 long and 0.030 wide in lateral aspect. Excretory vesicle without refractile concretions.

Host: Cerithium variabile.

Localities: Mayagüez (Punta Arenas) and Lajas (Parguera), Puerto Rico.

Cercaria caribbea XLIV
(Figs. 2, 3)

Specific diagnosis: Species of moderate size with the general characters of the group. Resting position with furcae spread. Caudal vesicle and tail stem with characteristic pigmentation; anterior portion of vesicle with dense longitudinal striae, probably muscles, followed by a region occupied by large cells with a pinkish-yellow to orange pigmentation. The posterior region of the caudal vesicle contains a reticulum of purplish-black pigment and approximately the middle third of the tail stem is characteristically marked by similar pigment. Eyespots 0.010 long and 0.014 wide; cuticula fairly thick. Oral vestibule 0.011 in depth; pharynx 0.022 to 0.025 long and 0.030 to 0.042 wide. Diameter of body retracted in caudal vesicle 0.110; detached body 0.275 long and 0.116 wide. Caudal vesicle 0.192 long and 0.144 wide; tail stem 0.48–0.49 from the attachment to vesicle to base of furcae, 0.080–0.085 in maximum width, and tapering toward each end. Furcae 0.192–0.233 long and 0.018 wide in dorsal aspect, 0.034–0.042 in lateral. Excretory vesicles filled with concretions of various sizes.

Host: Cerithium algicola.

Locality: Boquerón Bay, Puerto Rico.

Cercaria caribbea XLV
(Figs. 10, 11)

Specific diagnosis: This is the second largest species of the group. It swims for short periods and rests on the bottom with the furcae extended (Fig. 11). Tail with distinctive pigmentation; the caudal vesicle has 2 pairs of prominent black pigment masses confluent posteriorly with similar pigment around the capsule itself. A dorsal and ventral mass form a pair which is somewhat anterior to the less conspicuous lateral masses. A more diffuse golden-brown pigment occurs around the passage through which the body is withdrawn into the caudal vesicle. Similar brownish pigment is distributed along the edges of the tail stem while the furcae have a marginal reticulum of purplish-black pigment. This black pigment persists after the specimens are heat-killed or even fixed and prepared as whole mounts. Such treatment, however, causes contraction of circular muscles so that the shape of the tail is considerably altered from the living specimens in which the vesicle is more bell-shaped.

Caudal vesicle 0.308–0.320 long with a maximum width of 0.178; tail stem 0.575 long from attachment to base of furcae and 0.130 in maximum width at about middle. Furcae 0.384 long and 0.130 wide in lateral view. Retracted body 0.137–0.150 in diameter. Eyespots 0.011 long and 0.014 wide. Pharynx spherical, 0.031 in diameter.

Excretory vesicles filled with refractile spherical concretions of various sizes and opening into a slight depression at the posterior end of the detached body (Fig. 8). Flame cell formula of cercaria 2 [(4+4) + (4+4+4)] and of redia 2 [(4+3) + (3+3+4)].

Host: Cerithium algicola.

Locality: Boquerón Bay, Puerto Rico.

Cercaria caribbea XLVI
(Figs. 4, 5)

Specific diagnosis: This is the largest species of the group. It rests with furcae extended (Fig. 5). The caudal vesicle except the anterior protuberance is evenly pigmented a dark red color which is almost black in reflected light. So dense is this pigmentation that it is difficult to see the body capsule. No pigment is discernible in the tail stem.

Tail vesicle 0.384 long and 0.322 wide; tail stem 0.603 to 0.650 in length and 0.130 in width. Furcae 0.377–0.410 long and 0.103 wide. Detached body 0.355 long and 0.144 in maximum width at a level one-third from the anterior end. Body retracted in caudal capsule 0.178 in diameter. Pharynx 0.032 long and 0.036 wide; eyespots 0.009–0.011 long and 0.014–0.015 wide. Excretory vesicles filled with small refractile concretions.

Host: Cerithium algicola.

Locality: Boquerón Bay, Puerto Rico.

Cercaria caribbea XLVII
(Figs. 12, 13)

Specific diagnosis: This species is by far the smallest of the group. The caudal vesicle has a bright pink-red pigment and the tail stem a lighter pink coloration. The shape is considerably altered in the heat-killed specimens (cf. Figs. 12 and 13).

Diameter of the body capsule 0.071–0.072; caudal vesicle 0.178 long and 0.117 wide; length of tail stem 0.210, width in lateral aspect 0.062, and 0.041 to 0.047 in dorsal. Furcae 0.085–0.088

long, 0.028 wide in lateral aspect, and 0.020 in dorsal. Excretory vesicles filled with small refractile concretions.

Host: Cerithium floridanum.

Locality: Cabo Rojo (Sucia Bay), Puerto Rico.

Cercaria caribbea XLVIII

(Figs. 6, 7)

Specific diagnosis: Species of moderate size, tending to congregate at the surface; without noticeable pigment. The shape is not altered appreciably by heat-killing. The caudal vesicle is greatly enlarged, measuring 0.514 in length and 0.288 in maximum width; body capsule slightly longer than wide, measuring 0.142 long and 0.118 wide. Tail stem 0.493 long, 0.118 wide in lateral, and 0.082 wide in dorsal aspect. Furcae 0.220 in length, 0.048 wide in lateral, and 0.023 in dorsal view.

Body attached to tail but not retracted measures 0.350 in length. Pharynx 0.025 long and 0.028 wide; eyespots cup-shaped with a maximum width of 0.010.

Host: Cerithium algicola.

Locality: Cabo Rojo (Salinas Bay), Puerto Rico.

DISCUSSION

When La Rue (1926) proposed the order STRIGEATOIDEA, he included in it three major groups: the blood flukes, the strigeids, and the gasterostomes. At that time, fewer life histories were known than at the present and as additional ones were traced it became evident that still other groups belong in the STRIGEATOIDEA. Thus, have been added the CLINOSTOMATIDAE by Hunter and Hunter (1934), AZYGIIDAE by Szidat (1932), and BRACHYLAEMIDAE by Allison (1943). More recently, Cable (1953) assigned the FELLODISTOMATIDAE to the STRIGEATOIDEA. He traced the life history of a gymnophalline species and concluded that the GYMNOPHALLINAE, instead of belonging to the HETEROPHYIDAE or MICROPHALLIDAE, should be placed in the FELLODISTOMATIDAE. His view is supported by observations on several larval fellodistomatids and particularly the life history of *Fellodistomum fellis* as reported by Chubrik (1952). Thus, as additional life histories have been determined, the scope of the order STRIGEATOIDEA has been enlarged far beyond that originally conceived by La Rue. The present study adds still two other groups to the order, namely, the BIVESICULIDAE and the TRANSVERSOTREMATIDAE fam. nov., which is characterized below.

Of particular interest to the present study is the family AZYGIIDAE because its larvae, like those of the BIVESICULIDAE, are cystocercous cercariae with a forked tail. It is suggested that the term furcocystocercous be used to distinguish such larvae from other cystocercous forms, such as the cercariae of the GORGODERIDAE.

In describing the life history of *Hysteromorpha triloba,* a strigeid, Lutz (1931) noted the similarity of the cercaria to *Cercaria mirabilis* Braun and *C. anchoroides* Ward, both of which are furcocystocercous forms. He, accordingly, postulated an affinity of such cercariae to the strigeids and thus anticipated Szidat's (1932) conclusion that the cystocercous larvae of the AZYGIIDAE are basically furcocercous. Wesenberg-Lund (1934), Dickerman (1934), and Hussey (1943) presented further evidence supporting Szidat's view.

In the embryology of the excretory system, bivesiculid cercariae have much in common with the larvae of the cyathocotylids on one hand and those of the azygiids on the other. In complexity, bivesiculid cercariae are intermediate to the larvae of those well-known groups. While the furcae of the bivesiculid cercariae lack flame cells, the excretory system in the tail is much like that described by Hussey

(1943) for the larva of *Proterometra macrostoma* and by Komiya (1941) for *Cercaria shanghaiensis*. The chief difference between these azygiid larvae and the cercariae of the BIVESICULIDAE concerns the excretory tubules in the tail; in the AZYGIIDAE, the tubules fuse in the tail stem, either along its entire extent or with places where the embryonic tubules remain apart and form loops whereas fusion occurs at no place in the tail stem or body of the BIVESICULIDAE. An interesting aspect of the excretory pattern in the BIVESICULIDAE is the antero-posterior asymmetry with one more group of flame cells on the posterior collecting tubule than on the anterior, as compared to the symmetrical arrangement observed in most of the strigeid and cyathocotylid larvae.

Although the adults of the BIVESICULIDAE are similar to the MONORCHIIDAE, a careful examination shows that such resemblances are secondary and that the adult bivesiculids as well as their larvae have more in common with the strigeatoids. In several strigeatoid families, the genital pore is at the posterior end of the body and the cirrus sac is inverted, that is, directed posteriorly, as in the BIVESICULIDAE. In that family, however, the absence of a ventral sucker gives no point of reference for the location of the genital pore, which can be described only as being on the ventral side of the body and posterior to the mid-level. The excretory system of both larval and adult bivesiculids is entirely compatible with features of that system in the STRIGEATOIDEA. Furthermore, the few large eggs in the BIVESICULIDAE are similar to those found in many strigeatoids, and the development of the miracidium, so far as was determined, corresponds to that of the strigeids and schistosomes. The bivesiculids are, however, different from other members of the order in a number of respects, including the presence of an external seminal vesicle, the complete separation of the right and left halves of the excretory system, and the presence of a single testis, which, however, has two vasa efferentia.

In discussing the STRIGEATOIDEA and the larvae of that group, the peculiar type represented by *Cercaria pataliensis* Soparkar, 1924 and *C. koliensis* Olivier, 1947 should be included. These unusual larvae possess a body that is well-developed with adult characters far advanced and a furcocercous tail with unique processes at the base. Because of their resemblances to strigeid and schistosome cercariae, Olivier (1947) referred these larvae and *Transversotrema haasi* Witenberg, 1944 to the order STRIGEATOIDEA. The genus *Transversotrema* shows adult characters in precise agreement with those precociously developed in *Cercaria pataliensis* and *C. koliensis*. In describing *T. haasi*, Witenberg (1944) proposed without characterization a new subfamily, TRANSVERSOTREMINAE, which was not, however, assigned to any family, although he mentioned a resemblance to the MESOMETRIDAE Poche. However, such differences from the MESOMETRIDAE as the position of the ovary, location of the genital pore, cyclocoel gut, and presence of a ventral sucker in the TRANSVERSOTREMINAE, indicate that the subfamily does not belong in the MESOMETRIDAE. This view was expressed by Anantaraman (1948) who believed that *Transversotrema* may be more closely related to the CYCLOCOELIDAE than to the MESOMETRIDAE, although he acknowledged that developmental stages give little evidence of such an affinity. The writer is of the opinion that these peculiar forms constitute a natural group deserving the rank of family, and accordingly proposes that the subfamily TRANSVERSOTREMINAE of Witenberg be elevated to the rank of family with the following diagnosis:

TRANSVERSOTREMATIDAE FAM. NOV.

Diagnosis: Peculiar, transversely elongate, flattened trematodes with a ventral sucker but no oral sucker; mouth ventral, leading into a well-developed pharynx. Esophagus of moderate length; cyclocoel gut with wall uniformly scalloped. Cuticle with blunt scale-like spines in quincuncial arrangement. Genital pore on the anterior margin of the body; testes lobed, symmetrical, and situated within the ring of intestine; cirrus and cirrus sac absent, seminal vesicle free in the parenchyma; ovary lobed, near left testis; seminal receptacle absent, spermatozoa being stored in *receptaculum seminis uterinum;* Laurer's canal present. Vitellaria follicular, mostly extra-cecal around entire periphery of body but some follicles within the cecal ring as well. Eggs large, without filaments, and containing miracidia. Adults, parasites of fish, evidently both fresh-water and marine. Cercaria probably binoculate and furcocercous with adult characters well advanced in development. Excretory system of cercaria complex with many flame cells in the body and proximal tail stem, which bears also a pair of accessory caudal appendages at its base.

Type and only genus: *Transversotrema* Witenberg, 1944.

Probable larvae: *Cercaria pataliensis* Soparkar, 1924, *C. koliensis* Olivier, 1947.

SUMMARY

Six species of furcocystocercous cercariae found to develop in marine snails of the genus *Cerithium* evidently are larvae of species in the family BIVESICULIDAE. There apparently is no second intermediate host, the cercaria being eaten by the definitive host, a fish. In development of the cercaria, the right and left halves of the excretory system do not fuse either in the body or tail; thus, instead of a single excretory vesicle as in other trematodes, there is a pair of vesicles continuous with the separate caudal tubules which open at the tips of the furcae. The cercarial body has four groups of flame cells and the tail one group with half its flame cells in the caudal vesicle and half in the tail stem. In one species, the excretory formula is $2[(4+4)+(4+4+4)]$. The redia of all species has a cleft posterior end with the excretory pores at the tips of the lobes. In the redia of one species the excretory formula is $2[(4+3)+(3+3+4)]$. The BIVESICULIDAE, formerly associated with the MONORCHIIDAE, are more closely related to the STRIGEATOIDEA. In complexity, bivesiculid cercariae are intermediate to cyathocotylid and azygiid larvae and thus support the allocation of both the BIVESICULIDAE and AZYGIIDAE to the STRIGEATOIDEA. The subfamily TRANSVERSOTREMINAE is elevated to the rank of family, TRANSVERSOTREMATIDAE, to include *Transversotrema haasi* and as probable larvae, *Cercaria pataliensis* and *C. koliensis.*

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Explanation of Figures

(Unless otherwise stated, figures 1–13 are drawn to the same scale from heat-killed specimens under no pressure.)

Plate I

Fig. 1. *Cercaria caribbea XLIII,* dorsal (or ventral) view.
Fig. 2. *C. caribbea XLIV,* lateral view.
Fig. 3. *C. caribbea XLIV,* body not withdrawn into tail capsule.
Fig. 4. *C. caribbea XLVI,* lateral view.
Fig. 5. *C. caribbea XLVI,* shape and resting position of the living cercaria (free-hand).
Fig. 6. *C. caribbea XLVIII,* dorsal (or ventral) view.
Fig. 7. *C. caribbea XLVIII,* lateral view of tail stem.
Fig. 8. Posterior end of cercarial body showing the separate sphincters and pores of the two excretory vesicles (free-hand).
Fig. 9. Anterior end of cercarial body showing vestibule, cephalic glands, and their ducts (free-hand).

PLATE I

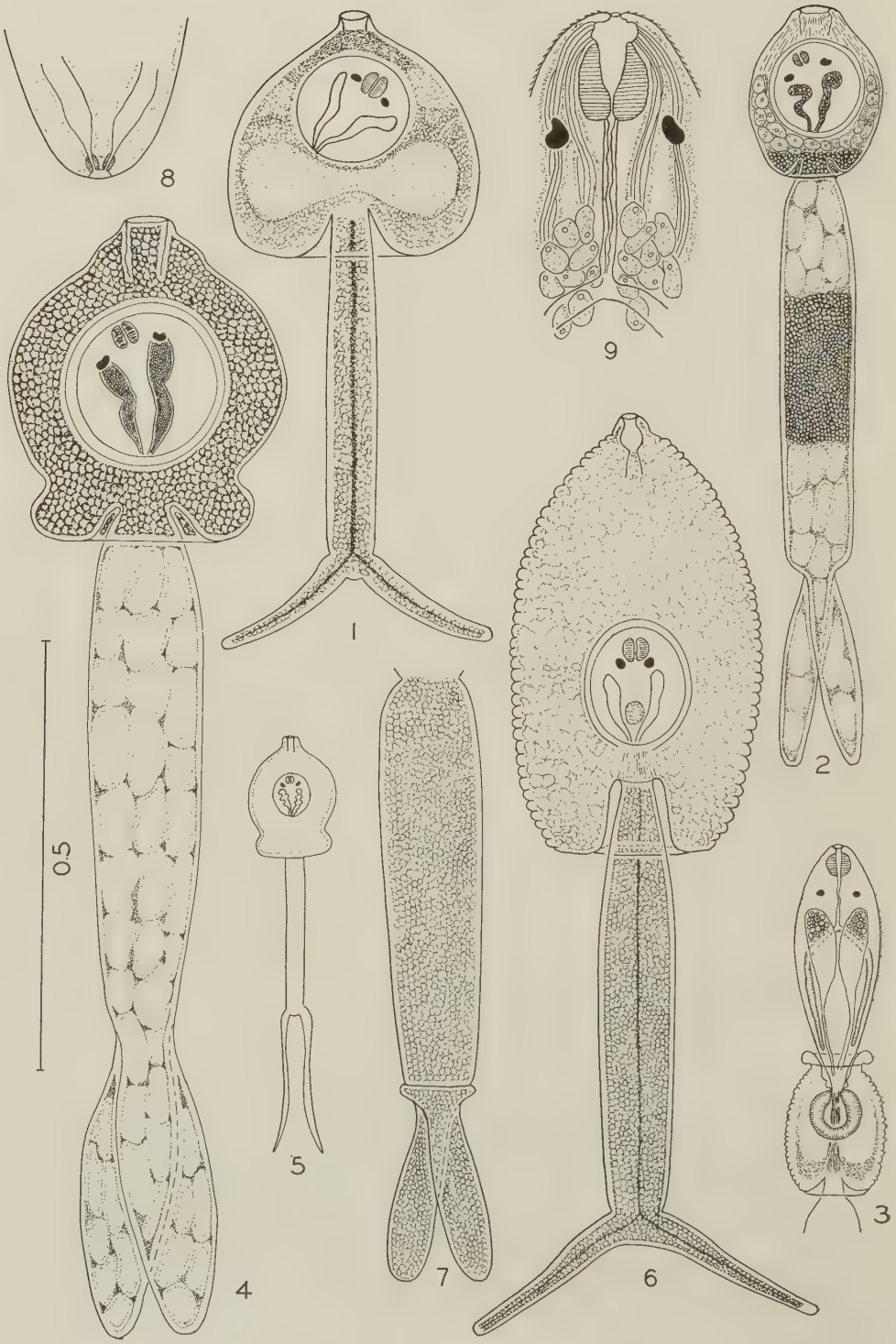


Plate II

Fig. 10. *C. caribbea XLV,* lateral view.
Fig. 11. *C. caribbea XLV,* shape and resting position of living specimen (free-hand).
Fig. 12. *C. caribbea XLVII,* lateral view.
Fig. 13. *C. caribbea XLVII,* lateral view of living specimen. To same scale as in Fig. 12.
Fig. 14. *C. caribbea XLV,* body and proximal tail region enlarged to show details of excretory system.
Figs. 15–19. *C. caribbea XLV,* developmental stages of the excretory system (free-hand).
Fig. 20. Young redia of *C. caribbea XLV,* showing cleft posterior extremity and resemblance to cercarial embryos (free-hand).
Fig. 21. Redia of *C. caribbea XLV,* showing the excretory system of one side (free-hand).

Plate III

All figures concern *Bivesicula hepsetiae.* Abbreviations:
CI—cirrus, *CS*—cirrus sac, *EG*—egg, *ES*—external seminal vesicle, *FC*—fertilization chamber, *GP*—genital pore, *IS*—internal seminal vesicle, *LC*—Laurer's canal, *MG*—Mehlis' gland, *OD*—oviduct, *OO*—oötype, *OV*—ovary, *PH*—pharynx, *TE*—testis, *US*—uterine seminal receptacle, *VE*—vas efferens, *VI*—vitellaria, *VR*—vitelline reservoir.
Fig. 22. Ventral view of stained specimen.
Fig. 23. Posterior end of body showing secondary invagination and sphincters of excretory vesicles (free-hand).
Fig. 24. Semi-diagrammatic sketch of reproductive system in ventral view.
Fig. 25. Miracidium within egg shell after two weeks of development.

Plate IV

Fig. 26. *Bivesicula hepsetiae,* longitudinal section through the pharynx of the adult.
Fig. 27. *Cercaria caribbea XLV,* longitudinal section through pharynx of redia.
Fig. 28. *C. caribbea XLV,* frontal section of advanced embryo.
Fig. 29. *C. caribbea XLV,* whole mount of redia photographed to show cleft posterior end.
Fig. 30. *C. caribbea XLV,* posterior end of redia showing location of germinal mass (arrow).
Fig. 31. *C. caribbea XLV,* frontal section of cercarial body showing genital primordia, excretory vesicles, and their sphincters.

PLATE II

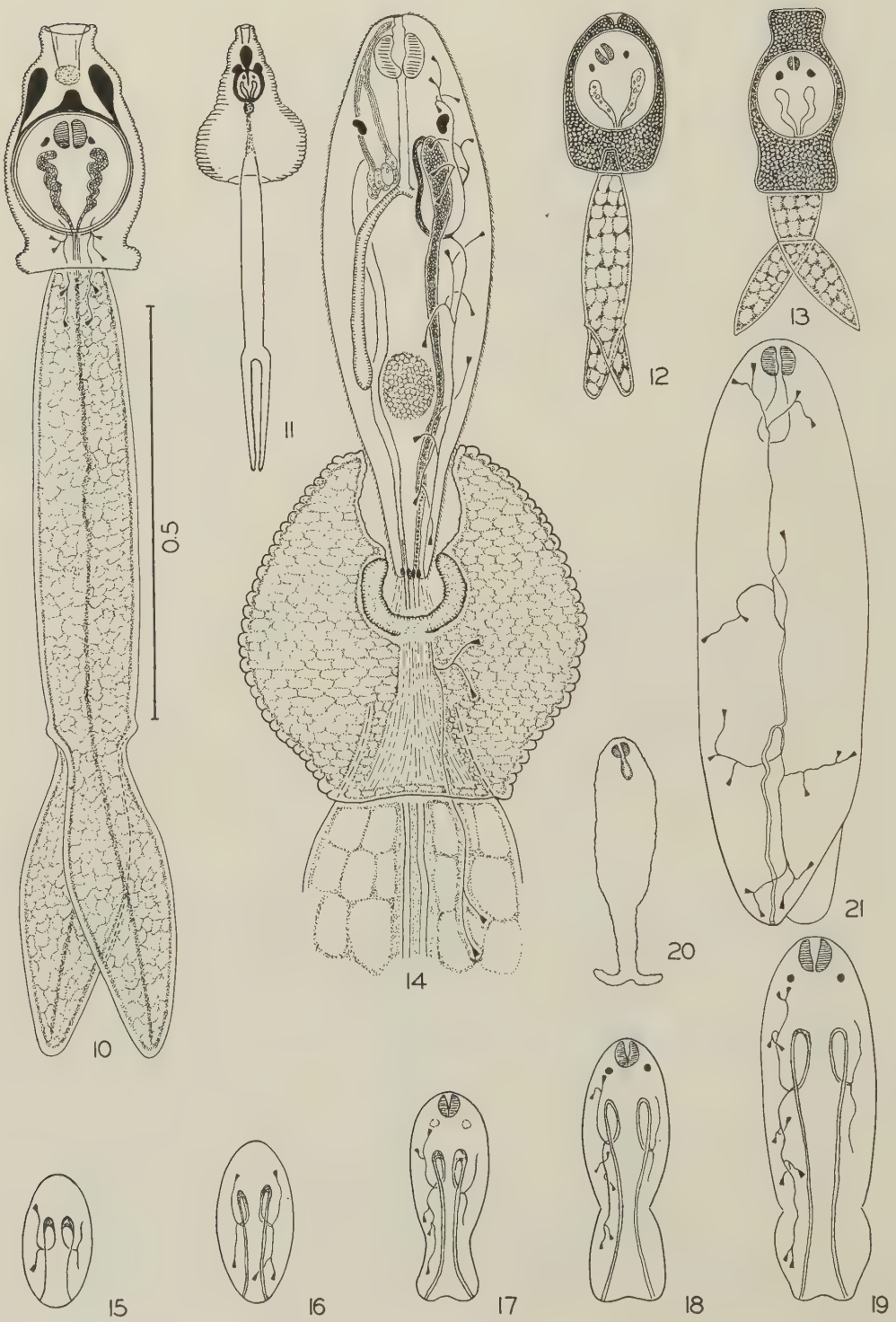
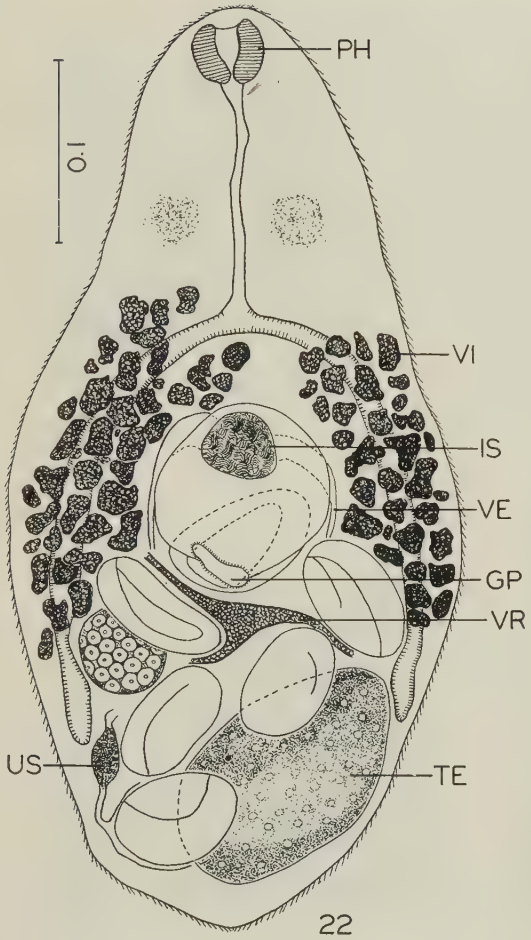


PLATE III



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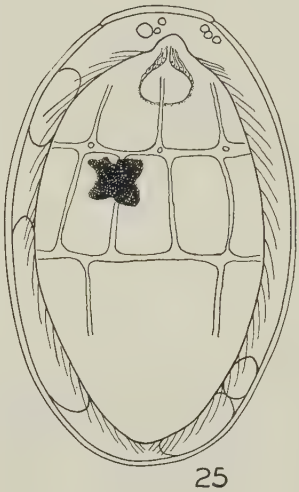

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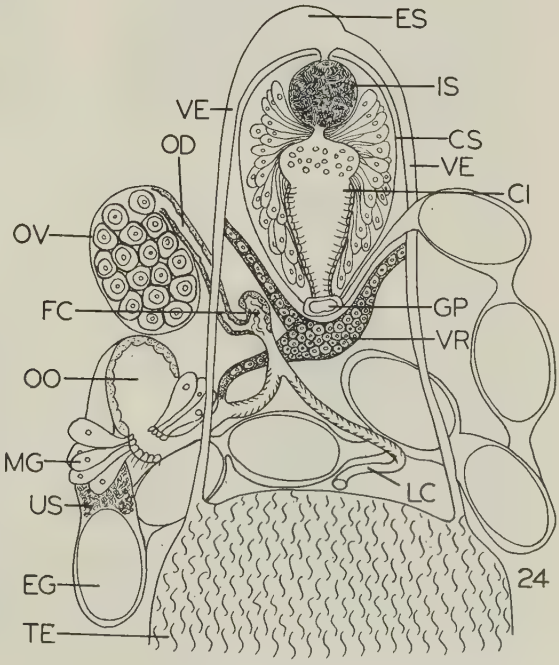

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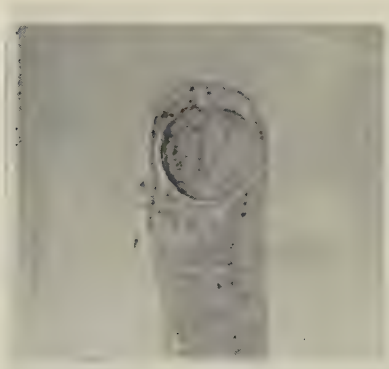
PLATE IV



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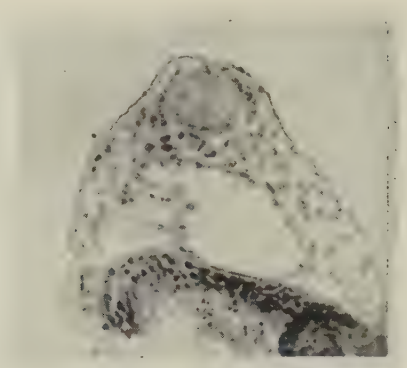

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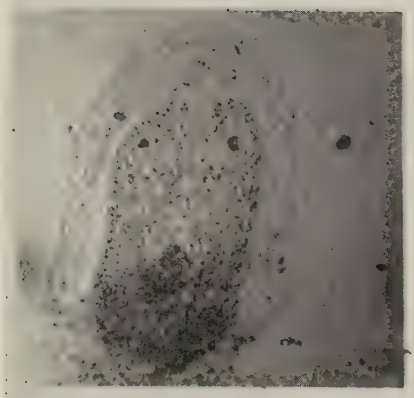

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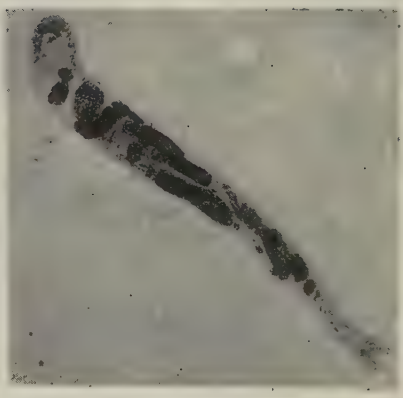

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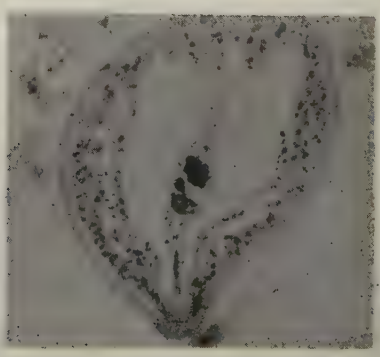

31

AMEBIC INFECTIONS IN DOGS*

Don E. Eyles, Frances E. Jones, John R. Jumper and Virginia P. Drinnon

Fecal specimens from a series of 143 dogs were examined for amebae by this laboratory as a part of a larger epidemiologic study of intestinal parasites in the Memphis, Tennessee area. The purpose of this study was to determine whether or not the dog might serve as a reservoir from which man could acquire infections, particularly of *Endamoeba histolytica,* in this region. Secondarily, the study was designed to provide additional information on the amebic parasites of the dog.

A review of the available literature shows that the dog has been found infected naturally with *E. histolytica* in a number of areas. Isolated instances of canine infection have been reported from India, Manchuria, Egypt, Panama, China, Indo-China, and the United States; however, studies which provide prevalence data are less frequent. In this country Swartzwelder and Avant (1952) reported 10 per cent of 70 dogs examined in New Orleans to be naturally infected. Frye and Meleney (1932) examined 22 dogs at New Hope in Jackson County, Tennessee but found no infections. No reports of other systematic investigations of dog populations were found.

Most reports indicate that in dogs only trophozoites are found. Only Andrews (1932) in Baltimore and Kubo (1936; as cited by Craig, 1944, p. 71) in Mukden have reported dogs passing cysts. Experimental infections have been described by a number of workers; in these cases, the dogs usually passed only trophozoites, although a liver diet was found by Kagy and Faust (1930) to stimulate the production of cysts.

Organisms identical to *Endamoeba coli* were reported by Andrews (1932) from a dog of Baltimore which was also infected with *E. histolytica.*

METHODS

The dogs which were examined for amebae were obtained from the Department of Pharmacology of the Medical School of the University of Tennessee. They originally came from the Memphis dog pound which serves both the City of Memphis and the adjacent rural sections in Shelby County. The individual dogs could not be differentiated as to point of origin.

For the examinations fecal matter was taken directly from the rectum at the time the dogs were sacrificed in connection with pharmacological experiments. These experiments were simple physiological exercises which probably had no effect on the parasite fauna of the animals.

Immediately after the specimens were obtained, a portion of each was planted on Nelson's medium for culture studies. In order to make it possible to prepare hematoxylin stained smears at a later date, another portion was mixed with polyvinyl alcohol-fixative (Brooke and Goldman, 1949). The remainder was examined directly in saline and in iodine solution after concentration.

Cultures were examined after 48 to 72 hours and any amebae found were stud-

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ied. In addition, preparations from these cultures were fixed and stained in iron-alum hematoxylin for further morphologic study. Subsequent passages were made, and the strains which grew well in culture were inoculated intracecally into rabbits. After two to four weeks the rabbits were sacrificed and examined to determine whether tissue invasion by amebae had taken place.

OBSERVATIONS

Prevalence of Endamoeba histolytica: Table 1 presents an analysis of the results of the series of examinations for this species both cumulatively and for the different techniques of examination. A total of 8.4 per cent of the dogs examined were found to be infected with amebae morphologically indistinguishable from *E. histolytica.*

Culture in Nelson's medium not only revealed the largest number of infections, but the few found by other methods were also found in culture. Direct comparison can be made of the techniques by consulting table 1 except that in the case of the polyvinyl alcohol technique only the final hundred specimens were examined.[1] In these the culture technique revealed nine infections as compared with the two found in stained smears prepared from the PVA preserved specimens.

TABLE 1.—*Prevalence of* Endamoeba histolytica *in dogs of the Memphis area*

Technique of examination	Number of dogs examined	Number infected	% Infected
Direct examination of feces in saline	138	1	0.7
Zinc sulfate concentration examined in iodine solution	138	0	0
Polyvinyl alcohol preserved specimen stained with iron-alum hematoxylin	100	2	2.0
Culture in Nelson's medium examined fresh after 48–72 hours	142	12	8.4
All methods combined	143	12	8.4

The finding of a single positive by direct examination in saline and only two by the examination of PVA-preserved specimens indicates that the infected dogs had only small numbers of parasites in the feces. In both instances trophozoites only were seen. The failure of the concentration technique to demonstrate the infections is possibly due to the failure of *E. histolytica* to produce cysts in the dog. Finally, the large number of infections demonstrated by the culture method indicates that *E. histolytica* of the dog multiplies very well in the medium employed.

For confirmation of the identification of the living trophozoites, smears from the cultures showing amebae were stained with iron-alum hematoxylin and examined. The culture diagnosis was verified by this procedure in all instances with one exception in which case, through an oversight, no stained preparations were made. In four instances cysts typical of *E. histolytica* were found in these preparations; chromatoid bodies characteristic of the species were readily demonstrated.

Although the parasites found were morphologically identical to *E. histolytica,* proof that they were physiologically identical was desired. To this end the capacity for tissue invasion was investigated by inoculating seven of the dog strains of amebae intracecally into rabbits. In five of the seven instances cecal lesions iden-

[1] The specimens preserved in polyvinyl alcohol-fixative were examined in the Protozoology Unit, Laboratory Branch, Communicable Disease Center in Atlanta, Georgia through the courtesy of Dr. Marion M. Brooke.

tical to those seen in rabbits inoculated with known human strains of *E. histolytica* were found. In all instances except one amebae were abundant at the level of the lesions.

Endamoeba coli: In the series of 143 dogs examined, two infections with organisms morphologically identical to *E. coli* were found. In one instance the fecal specimen contained large eight-nucleate cysts, and in the other instance trophozoites were recovered after culture. Except for the reported case of Andrews (1932) this is the only finding of this species in the dog of which we are aware.

Endolimax nana: In the case of one dog infected with *E. histolytica,* examination of the culture after 48 hours disclosed the presence of an ameba with the characteristics of *E. nana.* Study of the permanently stained preparation showed it to be morphologically indistinguishable from this parasite as found in man. To our knowledge, *E. nana* has not been reported previously from the dog.

In considering the findings in this study, it can not be overlooked that the prevalence rate for *E. histolytica* in dogs of this region was similar to that in humans while the rates for *E. coli* and *E. nana* fell far below those found in man. More favorable conditions for the multiplication of *E. histolytica* in culture may be a factor in these findings; however, it seems probable that the non-pathogenic amebae establish themselves very infrequently in dogs.

DISCUSSION

The possible role of the dog in the epidemiology of *E. histolytica* has been considered by others, and the consensus has been that the dog could not be important in transmission of the parasite because of the infrequency of infection and particularly because cysts are not ordinarily passed in the feces. The report of Swartzwelder and Avant (1952) and the present report indicate that in the southern region the infection rate of *E. histolytica* in dogs is not greatly dissimilar to that in man.

The belief that cysts are not frequently passed is supported by this study. However, transmission of *E. histolytica* by ingestion of the trophozoite forms has been demonstrated by several investigators. It possibly occurs more often than is generally thought.

On the contrary, the present study indicates that infected dogs frequently pass so few amebae as to make their detection by direct method impossible. This would mitigate against the dog's serving as a source of infection, but considering the very intimate association which frequently exists between children and dogs, the possibility of occasional infections being acquired from the dog cannot be discarded. Likewise dog to dog transmission may occur, but it would appear more likely that the dog obtains the infection by the ingestion of human feces.

The results of the present study are of especial interest from the point of view of determining the relative efficiency of the different techniques employed in the detection of the infections. In the case of the dogs, the parasite picture is not confused by the frequent occurrence of amebic species which can be mistaken for *E. histolytica.* Under these circumstances, the culture technique was found to reveal many times as many infections as any of the other methods employed. Since there is little or no confusion as to the identification of the organisms growing in the cultures, we can say with certainty that the culture technique is many times more efficient than the others used in our hands.

Of course, the failure of the dog to pass cysts under most circumstances prevents a fair comparison between concentration and the other techniques in the present case; however, the efficiency of the direct examination of feces in saline and the examination of the PVA preserved specimens, and the efficiency of the culture method in the detection of small numbers of trophozoites are directly comparable.

SUMMARY AND CONCLUSIONS

One hundred forty-three dogs from the Memphis, Tennessee dog pound were examined for intestinal amebae with the following results: *Endamoeba histolytica* infections were found in 12 or 8.4% of the dogs, *Endamoeba coli* in two of these and *Endolimax nana* in a single dog.

Culture of the specimens was the most successful method of examination in uncovering these infections. The limited numbers revealed by the other techniques used indicated that cysts of amebae are almost never passed and trophozoite forms are passed in very small numbers in the feces of dogs.

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THE RELATIONSHIP OF MALE WORMS TO THE SEXUAL DEVELOPMENT OF FEMALE *SCHISTOSOMA MANSONI*[1]

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REVIEW OF LITERATURE

It is now generally accepted that in the schistosomes all of the cercariae resulting from a single miracidium are of the same sex. Various investigators (Katsurada, 1914; Tanabe, 1919; Kasama, 1921; Suyemori, 1922; Faust and Meleney, 1924; Faust, 1927; Severinghaus, 1928; Sagawa, Ogi, and Sumikoshi, 1928; Tanaka, 1929; Lee, 1932; and Suguira, 1933) have shown that when a single snail is used

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for infecting animals with *Schistosoma japonicum* the resulting infection is usually unisexual. All of these investigators undoubtedly used naturally infected snails. Similar results have been obtained with *S. mansoni* infections obtained from naturally infected *Australorbis glabratus* by Mayer and Pifano (1942) and Jaffe, Mayer and Pifano (1945). Maldonado and Herrera (1949) have emphasized the point that in *S. mansoni* infections not all naturally infected snails harbor unisexual infections and have shown that in highly endemic areas both sexes may predominate.

The possibility of the existence of unisexual schistosome infections in the mammalian host has led to numerous investigations on the influence of one sex of schistosome on the sexual maturity and body growth of the other sex in the final host. Severinghaus (1928) Sagawa, Ogi and Sumikoshi (1928) and Lee (1932) have shown that in unisexual infections of *S. japonicum* both sexes fail to develop normally. The male worms in a unisexual infection develop to sexual maturity and produce spermatozoa but they grow more slowly and are smaller than male worms from bisexual infections. The females of this species in a unisexual infection never reach sexual maturity and Severinghaus (1928) showed that they reach only about one-fifth of the body length of females paired with males in a bisexual infection. Severinghaus (1928) showed that the females would remain in this undeveloped state for at least 268 days but if at any time male-producing cercariae were introduced into the animal harboring these immature females, the females would rapidly complete their normal development in size, form and production of germ cells. He cited one striking example where unisexual females were 128 days old when male-producing cercariae were introduced into the host. Nineteen days later the females had increased greatly in size, although they were still a little smaller than normal, and the reproductive organs were fully developed.

Vogel (1941), Moore, Yolles and Meleney (1949b) and Maldonado and Herrera (1949) have shown that in unisexual *S. mansoni* infections the females do not attain full body size nor do they reach sexual maturity. Vogel (1941) found that the females of *S. mansoni* in a unisexual infection reach a little less than one-half the body length of specimens mated with males, and that they remain small and sexually immature for at least 10 months. Vogel also found that the male *S. mansoni* in unisexual infections, in contrast to *S. japonicum* males, develops to full body size and sexual maturity in the absence of female worms.

Brumpt (1936b) noted that in unisexual female infections of *S. haematobium, S. bovis* and *S. mansoni* the worms did not mature.

Vogel (1941) also infected mammalian hosts with the male *Schistosoma* of one species and females of another in the following crosses: *japonicum* ♂ × *mansoni* ♀; *mansoni* ♂ × *japonicum* ♀; *haematobium* ♂ × *mansoni* ♀; *mansoni* ♂ × *haematobium* ♀; and *japonicum* ♂ × *haematobium* ♀. He found that in each case the males copulated with the females of the different species and stimulated their development to sexual maturity. The growth of the females was also stimulated but they did not attain the full size of the females mated with males of their own species. In some instances the growth of the male worms was stimulated and in others it seemed to be retarded. Short (1948) performed similar crossing experiments using males of *Schistosomatium douthitti* and females of *Schistosoma mansoni*. Although the females in these experiments remained small many of them attained sexual maturity. Thus it seems that the influence of the male schistosome on the growth

and sexual development of the female is not species specific (Vogel 1941), nor is it genus specific (Short 1948).

Brumpt (1936a) observed that the females of *Bilharziella polonica* developed normally in the absence of male worms and laid eggs which were normal in appearance but did not contain miracidia. Short (1947, 1952) reported the same phenomenon in *Schistosomatium douthitti* but observed that the eggs contained normal miracidia. Short noted no delay in the time required for the attainment of sexual maturity in the unisexual female infections by comparing their development with females mated with males in a bisexual infection. This parthenogenetic reproduction has not been observed in either *Schistosoma japonicum* or *S. mansoni.*

Vogel (1941), discussing the influence of one sex of schistosome on the other, concluded that neither the nature of the influence nor the manner in which it is exerted is known. The purpose of the experiments reported here was to investigate further the relationship of the male *S. mansoni* to the sexual development of the female worm.

MATERIALS AND METHODS

Our present colony of *Australorbis glabratus* was started from snails received from Drs. J. Oliver-Gonzales and J. F. Maldonado of the School of Tropical Medicine in Puerto Rico in 1949. Unisexual infections in the snail host were obtained by exposing snails 1–1.5 cm. in diameter to single miracidia of *Schistosoma mansoni.* The snails were exposed individually in 5 ml. beakers. After exposure all of the snails exposed on the same day were placed together in a battery jar aquarium containing sand and *Elodea,* until cercariae were first observed in that group of snails. The snails were then set up individually to determine which of the snails were shedding cercariae and the shedding snails were isolated in a battery jar aquarium and identified by a numbering system. No animal exposures were made from these individual snails until they had been isolated at least 24 hours to prevent any contamination of cercariae from other shedding snails which might have been carried over into the isolation aquarium. Albino mice were exposed percutaneously each day to 150 cercariae according to the method described by Moore, Yolles and Meleney (1949a). Four weeks after exposure one mouse from the first group of animals exposed to cercariae from each individual snail was autopsied to determine the sex of the infection harbored by the snail used for the infection. Before beginning any experiments involving unisexual female infections, the mice were allowed to harbor this infection for at least 11 weeks. From previous experiments (Moore, Yolles and Meleney, 1949a) we had determined that all of the female worms, in a normally balanced bisexual infection, would be sexually mature at this time. Worms were recovered from the infected animals by the perfusion technique of Yolles, Moore, DeGiusti, Ripsom and Meleney (1947). A few worms from each animal were stained with neutral red and examined microscopically while living. Observations were made on the degree of development of the vitellaria and ovary and the presence or absence of an egg in the oötype as well as general observations on the size of the worms. The remainder of the worms recovered from the experimental and control animals were fixed in Formal-Acetic Acid-Alcohol (F. A. A.) using the coverslip method. The worms were stained with Mayer's Carmalum and mounted as toto-mounts for further study and measuring. Measurements of the

length of the worms were made by projecting the image of the worm (150 × magnification), on a white cardboard attached to the wall in a dark room. A map reader, which was calibrated by projecting a slide micrometer image on the screen, was used to make the length measurements. Care was taken to make the map reader tracing along the center of the projected image of the worm. Measurements of the width of the worms in the region of the ovary, and of the length and width of the ovary, were made with an ocular micrometer.

Fecal examinations were performed at regular intervals on all experimental and control animals using the direct smear and saline sedimentation-hatching techniques. At the time of autopsy of the infected animals tissue press examinations were made of liver, lung, spleen, duodenum, jejunum, ileum and colon to determine the presence or absence of eggs.

EXPERIMENTAL DETAILS AND RESULTS

Experiment I

The purpose of this experiment was to determine the rate of development and sexual maturation of female *S. mansoni* worms in a unisexual infection after exposure of the host animal to male-producing cercariae. The mice harboring the unisexual female infection were exposed to 150 male-producing cercariae when the female infection was 11 weeks old. These mice were autopsied at 1 week, 2 weeks and thereafter at ½-week intervals through 8 weeks after exposure to male-producing cercariae. The control animals consisted of a group of mice harboring unisexual female infection, of the same age as the female infections in the experimental animals, which were not exposed to male-producing cercariae. Control animals were autopsied at weekly intervals throughout the experiment.

The sexually immature female *S. mansoni* (Fig. 1) from a unisexual infection of 11 + weeks duration is quite different from the sexually mature female worm. The measurements used in the following description of the unisexual female *S. mansoni* were obtained from the 81 control worms in this experiment. The female worms from a unisexual infection average 4.38 mm. (range 2.73–6.03 mm.) in length and 0.122 mm. (range 0.091–0.202 mm.) in width in the region of the ovary. The living worms are flatter than the sexually mature females and the edges of the body tend to roll ventrally forming a pseudo-gynecophoral canal. The somatic nuclei are quite compact and it appears that there are more nuclei per unit area in the immature female than in the sexually mature female. The fundament of the vitelline glands is visible as small clumps of cells located on both sides of, and in close proximity to, the cecum. The vitelline duct is seldom discernible posterior to the ovary in toto-mounts but in well differentiated specimens it can be seen between the anterior end of the ovary and the oötype. The cellular portion of the ovary averages 0.153 mm. (range 0.091–0.247 mm.) in length and has a loose undulant shape. The ovary is nearly uniform in width throughout the length of the cellular region, and, at the posterior end of the cellular region, averages 0.037 mm. (range 0.026–0.058 mm.) in width. The oögonial cells are small, compact and of uniform size throughout the length of the ovary. There is no evidence of maturation of the oögonial cells or of ovulation. The cellular elements of the ovary end rather abruptly forming a transverse line. The delimiting membrane of the ovary continues posteriorly to form a funnel-shaped chamber about 0.032 mm. long (Fig. 2)

which is contiguous with the oviduct. In the sexually mature female this funnel-shaped chamber becomes filled with ova, as they separate after ovulation, and undergoes considerable expansion (Fig. 3). The convolutions and recurving of the oviduct can be traced posterior and anterior to the ovary in the sexually immature worms.

Faust, Jones and Hoffman (1934) described a true seminal receptacle for *S. mansoni* as a blind pouch arising from the oviduct soon after its emergence from the ovary. We have not been able to confirm this observation. Our observations are in accordance with those of Severinghaus (1928) for *S. japonicum* who described the normal condition as a fertilization chamber, which is an enlarged portion of the oviduct, just behind the ovary, in which spermatozoa accumulate. He did see one worm with a true seminal receptacle, and a few worms with intermediate stages which he interpreted as representing the transition from the true seminal receptacle to the true fertilization chamber. During the microscopic examination of sexually immature and sexually mature worms from our experiments, in which

TABLE 1.—*Average measurements (mm.) of worms from Experiment I: Mice harboring 11 week old unisexual females exposed to male cercariae*

Age of Worms in weeks				Experimental ♀		Experimental ♀ ovary						Control ♀		Control ♀ ovary	
♀	♂	No. Exp. Mice Used	No. Exp. ♀ Meas.	Length	Width	Length	Width	No. ♂ Meas.	Exp. ♂ Leng.	No. Control Mice Used	No. Control ♀ Meas.	Length	Width	Length	Width
12	1	1	3	4.64	0.113	0.167	0.032			1	8	4.06	0.108	0.165	0.034
13	2	2	21	5.44	0.106	0.206	0.033			1	16	3.39	0.134	0.117	0.039
13½	2½	2	9	4.02	0.128	0.165	0.034								
14	3	2	15	3.93	0.108	0.159	0.037	21	0.47	1	10	4.29	0.117	0.149	0.041
14½	3½	2	11	3.67	0.126	0.144	0.034	20	0.80						
15	4	2	16	4.26	0.119	0.172	0.035	22	2.50	1	11	4.79	0.119	0.173	0.037
15½	4½	2	15	5.39	0.105	0.195	0.036	12	2.86						
16	5	2	8	5.59	0.091	0.177	0.050	14	3.75	1	15	4.65	0.124	0.159	0.039
16½	5½	2	7	5.60	0.137	0.231	0.068	10	5.65						
17	6	1	3	6.41	0.163	0.277	0.076	16	5.02	2	16	5.10	0.118	0.174	0.034
17½	6½	2	13	6.65	0.125	0.252	0.073	25	5.79						
18	7	1	4	5.99	0.145	0.232	0.091	9	4.60	1	3	4.52	0.130	0.134	0.034
18½	7½	2	4	8.09	0.142	0.271	0.091	10	6.47						
19	8	1	10	6.36	0.137	0.276	0.074	16	7.85	1	2	3.90	0.120	0.101	0.035

the oviduct could be traced in its entirety without being masked or hidden by a yolk-filled vitelline duct we could find no evidence of a seminal receptacle as described by Faust *et al.* (1934). The fertilization chamber becomes apparent as such only after it becomes filled with spermatozoa.

The oötype appears to be completely developed in the immature worm. When living worms were examined the oötype was actively expanding and contracting. The uterus is completely developed and in living specimens peristaltic movements of the uterus were observed. It is interesting to note that the contents of the ceca of the immature females is either fresh red blood or a light brownish substance mixed with the fresh red blood, while in the mature females the ceca are invariably filled with black hematin, and fresh red blood is seldom seen. It is probable that the immature females do not feed as voraciously as the mature worms.

In Experiment I no evidence of sexual development in the female worms was observed up to 4 weeks after exposure of the mice to male-producing cercariae. It can be seen in Table 1 that during this period of time the measurements of the female worms from the experimental animals are essentially equal to those from the control animals. An egg shell was observed in the anterior tip of the uterus of one female

worm recovered from an experimental mouse 2½ weeks after infection. The presence of this egg shell, which did not enclose an ovum, might be considered as evidence of some activity of the vitelline and Mehlis' glands. The ovary of this worm showed no evidence of maturation. At this time most of the 2½ week old male worms in the animal were in the immature kappa stage of development as described by Faust *et al.* (1934).

Four weeks after the exposure of the experimental animals to male-producing cercariae there was no evidence of sexual development of the female worms. At this time the male worms averaged 2.5 mm. in length. Fifteen of the 18 male worms which could be examined critically showed well developed testes but the seminal vesicle was empty. The remaining three worms were not as well developed and the testes could not be seen.

Worms were first found in copula 4½ weeks after exposure of the animals to male-producing cercariae. At this time there was essentially no change in the female worms. In two of the females the fundament of the vitellaria was slightly larger but there was no evidence of any secretory activity of these glands. There was evidence of some activity of the oögonial cells in the posterior region of the ovary. These cells were larger than the other ovarian cells and the nucleus was less compact, showing a network of chromatin. In one worm a few cells could be seen protruding into the funnel-shaped posterior portion of the ovary. No spermatozoa could be found in either of these worms. The male worms at this time averaged 2.86 mm. in length. Of the 13 male worms examined, 3 were small and showed no evidence of testes, whereas in the other 10 the testes and seminal vesicle were well developed. Of these 10 males, 3 had an empty seminal vesicle, in 6 the seminal vesicle was packed with cells which were interpreted as being spermatids, and in the other it contained a few spermatozoa as well as spermatids.

At 5 weeks after the exposure of the experimental animals to male cercariae many changes had taken place. More worms were found in copula and in one of the two animals autopsied a very immature or infertile egg was seen in the tissue press examination of the liver. Fecal examinations on these animals did not reveal any eggs. Nine female worms were examined critically; 8 of these had a normal appearing egg either in the oötype or uterus. In 2 of the worms an ovum could be seen in the egg. In the others the collapse of the egg shell during the mounting process made it impossible to determine whether or not the egg contained an ovum. In one worm two abnormal eggs were seen, neither containing an ovum. Considerable development had taken place in the ovary of all worms examined. The cells in the posterior third of the ovary were round and large and the chromatin material in the nucleus was quite compact as compared with the diffuse network of chromatin seen in the cells at 4½ weeks. These cells, probably mature ova, were loosely packed into the funnel-shaped portion of the ovary and in several instances a single ovum could be seen at the entrance to the oviduct or in it.

The presence of ova in the funnel-shaped chamber had begun to influence the width of the posterior region of the ovary. The average width of the posterior portion of the ovary at this time was 0.050 mm. (Table 1) as compared with 0.039 mm. for the 5 week controls and 0.036 mm. for the worms recovered from the experimental animals at 4½ weeks. Although no spermatozoa were seen in the females examined, this does not mean that they had not been inseminated. It is conceivable

that only a few spermatozoa are introduced into the female at first and it would be difficult to detect their presence in toto-mounts. Yolk was present in the vitelline duct of all worms examined and the vitellaria in the anterior portion of the vitelline region were quite well developed. The nuclei of the vitelline gland cells were large and the chromatin suggested a state of active multiplication. Growth and development of the vitellaria begins in the most anterior glands and progresses posteriorly. In all of these worms there was little or no evidence of the development of the vitellaria in the posterior half of the vitelline region.

At this time the male worms averaged 3.75 mm. in length. Of 14 which were examined, 2 were small and undeveloped, and testes could not be seen. In the other 12 the testes were well developed. Five of these 12 worms had only spermatids in the seminal vesicle, the remaining 7 had spermatozoa. The number of spermatozoa in the seminal vesicle ranged from a few associated with many spermatids, to many without spermatids. The female worms from the control animal at this time were all typical sexually immature worms.

In the experimental animals autopsied 5½ weeks after exposure to male-producing cercariae, no eggs had been found in the feces during life. They were found in the press preparations of the liver, but not of the intestine. Ten female worms were examined; 7 of these had a normal egg in the oötype and 2 others had ova in

TABLE 2.—*Linear regression coefficients of measurements on experimental and control worms from Experiment I*

	Length of worm	Width of worm	Length of ovary	Width of ovary
Experimental *b*	0.395 mm.	0.00470 mm.	0.0160 mm.	0.00836 mm.
Control *b*	0.227 mm.	0.00148 mm.	0.00385 mm.	0.00061 mm.

the oviduct. Spermatozoa were easily detected in the fertilization chamber of 6 of the 10 females, the number of spermatozoa varying from a scattered few to many. In some of the worms with many spermatozoa, they could be seen in the oviduct for about half the length of the ovary, the greatest concentration of spermatozoa always being in the fertilization chamber close to the ovary. There was an increase in the number of ova in the posterior region of the ovary, causing an increase in the width of this region. The average width of the posterior region of the ovary was 0.068 mm. The development and functioning of the vitelline glands had progressed posteriorly so that approximately two-thirds of the vitelline region was developed. The male worms at this time averaged 5.65 mm. in length and all 10 male worms examined had spermatozoa in the seminal vesicle.

From 6 through 8 weeks after the introduction of male-producing cercariae into the experimental animals there was no great change in the sexual development of the worms. Both males and females continued to increase in length. There was essentially no change in the width of the females or the length of the ovary (Table 1). The posterior region of the ovary continued to increase in width as more ova became mature and migrated into the funnel-shaped chamber awaiting entrance into the oviduct. At six weeks eggs were found in the feces and in tissue-press preparations of the liver, duodenum, jejunum and ileum. They were not seen in the colon until 7 weeks. The vitellaria were developed throughout the entire length of the vitelline region at 6 weeks. The female worms from the control animals were still

sexually immature at the termination of the experiment when they were 19 weeks old.

Table 2 shows the linear regression coefficients representing the regressions of the length and width of experimental and control females and the length and width of the ovary of experimental and control females against the age of the worms in weeks from 12 to 19 weeks. For each of the four variables, the experimental group showed a significantly higher regression coefficient than the control group.

These linear regression coefficients are merely expressions of the average growth in millimeters per week of age. Since the rate of growth is not likely to be constant throughout, the values of these coefficients may be greatly influenced by unequal numbers of worms examined at various periods of observation. The full analysis of these data should have allowed for the fact that the worms from the same mouse will tend to resemble each other. The above estimates and tests were made as if this were not so, therefore this one reservation must be considered when stating that for each of the measurements made, the regression coefficients for the experimental worms were significantly higher than those for the control worms.

Experiment II

Girges (1934) proposed the theory that the presence of a unisexual male infection of *S. mansoni* prevents the subsequent development of any female worms to which the host may be exposed. The purpose of this experiment was to determine whether or not female worms were capable of developing in mice harboring a unisexual male infection and if so to determine the rate of development and sexual maturation of the female worms. In contrast to the female worms, the male worms reach sexual maturity in a unisexual infection. The testes become fully developed and the seminal vesicle is filled with spermatozoa.

The experimental mice harboring a unisexual male infection of 35–36 weeks duration were exposed to female-producing cercariae. The animals were autopsied 3 times a week from 3 through 8 weeks after exposure to females. The worms recovered from these animals were not measured because many of the worms contracted and became twisted during fixation.

The worms were first found in copula 4-4/7 weeks after exposure to females. At this time only 2 of the 11 females recovered from the mouse autopsied, were in copula. They were in the *pi* stage of development. Five weeks after the introduction of females approximately half of the females were in copula. None of them were sexually mature but in general the worms residing in the gynecophoral canal of the males were larger and more advanced than the worms which were not yet in copula. Two days later (5-2/7 weeks) two mice were autopsied. Each mouse harbored 13 pairs in copula and only 4 and 5 female worms respectively which were not in copula. Three females had reached sexual maturity; spermatozoa were seen in the fertilization chamber of the oviduct, yolk was apparent in the vitelline duct and the posterior funnel-shaped chamber of the ovary contained a few ova. Eggs were not seen in the oötype or uterus of two of these females. In the third female 4 abnormal eggs without ova were seen in the uterus. The remainder of the female worms from these mice were all sexually immature, ranging from one worm in the *mu* stage to the typical appearance of a sexually immature female from a unisexual

infection. Only a few female worms were recovered from the mouse autopsied at 5-4/7 weeks and all were sexually immature.

Six weeks after the introduction of female-producing cercariae a total of 47 female worms from 2 mice were examined. Ten of these were still sexually immature; 4 were approaching sexual maturity, as evidenced by the beginning of development of the vitelline glands, the beginning of ovulation and the migration of ova into the funnel-shaped chamber at the posterior end of the ovary; 33 worms were sexually mature, showing spermatozoa in the fertilization chamber, yolk in the vitelline duct and a normal egg containing an ovum in the oötype or uterus. At this time the tissue press preparations and the feces were negative for eggs. From 6 through 8 weeks after the introduction of female worms the only changes observed were the sexual maturation of all females at 7 weeks, presence of eggs in the liver, jejunum, ileum and colon at 6-4/7 weeks, and the appearance of eggs in the feces at 7-2/7 weeks.

Experiment III

In order to determine in detail the normal sexual development of the female *S. mansoni* in a bisexual infection where the males and females are of the same age,

Table 3.—*Average measurements (mm.) of worms from Experiment III: Mice exposed to males and females simultaneously*

Age of worms weeks	No. mice used	No. meas.	Females				Males	
			Worm		Ovary		No. meas.	Length
			Length	Width	Length	Width		
5	1	16	3.54	0.145	0.114	0.042	7	3.74
5 2/7	1	5	3.94	0.122	0.128	0.039	12	4.31
5 4/7	1	10	5.03	0.133	0.181	0.056	18	4.47
6	1	10	5.21	0.108	0.240	0.066	7	5.54
6 1/2	1	7	6.48	0.126	0.240	0.071	8	6.84
7	1	13	7.67	0.141	0.296	0.095	12	6.53
8	1	13	7.26	0.162	0.266	0.096	8	7.04

12 albino mice were exposed individually to 150 cercariae from our general stock of *A. glabratus*. In order to insure a well balanced infection pooled cercariae from at least 20 snails were used. The animals were autopsied at half-week intervals from 3 to 4 weeks, 3 times a week from 4 to 6 weeks, and at half-week intervals from 6 to 8 weeks after exposure.

From 3 to 4-4/7 weeks after infection the worms recovered were very small and in many instances it was difficult if not impossible to determine the sex. In the most advanced males the testes were observed as small compact groups of large nucleated germinal cells. None of the worms recovered were in copula.

Worms were first found in copula at 5 weeks (2 pairs out of a total of 36 worms recovered). At this time the female worms were all sexually immature and were identical with the typical sexually immature female from a unisexual infection. The females averaged 3.54 mm. in length, 0.145 mm. in width (Table 3). The male worms presented a more varied developmental picture. They average 3.74 mm. in length (range 1.87 to 4.90 mm.). Seven male worms were examined in stained toto-mounts. One had spermatozoa and spermatids in the seminal vesicle, 3 had only spermatids and 3 had neither spermatozoa nor spermatids.

At 5-2/7 weeks there were 4 pairs in copula out of a total of 25 worms recovered. There was essentially no change in the development of the female worms,

they were all sexually immature. Twelve male worms were examined; the testes were well developed and spermatids were seen in the seminal vesicle of all except 3 worms.

At 5-4/7 weeks there was an increase in the number of worms in copula; 11 pairs out of a total of 33 worms recovered. At this time the female worms had matured considerably. A total of 11 females were examined critically; 5 of these had functional vitellaria, the remainder showed some evidence of growth of the vitelline glands but had no yolk in the vitelline duct. Spermatozoa were seen in the fertilization chamber of 5 worms and three worms had a normal egg containing an ovum in the oötype. A few eggs were seen in the tissue press preparations of the liver and ileum. No eggs had been found in the feces of this animal. Eighteen male worms were examined; 8 were undeveloped sexually; 5 had only spermatids in the seminal vesicle and 5 had both spermatids and spermatozoa.

At 6 weeks after infection, 8 pairs of worms out of a total of 31 worms recovered were in copula. A total of 13 females were examined in stained toto-mounts; 7 were fully mature, 6 were sexually immature and were similar to female worms from a unisexual infection. A total of 9 male worms were examined; 6 were fully mature with only spermatozoa in the seminal vesicle; one had spermatozoa and spermatids; one had spermatids only and one was sexually immature. By 7 weeks all of the worms were mature, eggs were seen in the tissue press preparations of liver, duodenum, jejunum, ileum and colon. Eggs were found in the feces for the first time 7 weeks after exposure.

Experiment IV

Severinghaus (1928) suggested that the male *S. japonicum* might produce a hormone which stimulated the female worms to complete their sexual development. Sagawa, Ogi and Sumikoshi (1928) also thinking along the lines of hormonal influence considered that the hormone might be secreted into the blood of the host. In their experiments they used rabbits harboring a unisexual infection of *S. japonicum* and gave these animals intravenous injections of a saline extract of the worms; in another experiment they injected the serum of rabbits harboring bisexual infections into animals harboring unisexual infections. Both experiments produced negative results.

Our purpose in performing this experiment was to determine if any sexual development of immature females in a unisexual infection could be stimulated by the injection of a particulate suspension of male worms into the experimental animals.

Mature male worms were collected from unisexual and bisexual infections of mice, hamsters and rabbits. After the male worms were collected by perfusion they were washed in several changes of physiological saline and placed in a vacuum desiccator. A total of 6,137 male worms were collected and desiccated. The dried whole worms were pulverized with an agate mortar and pestle and the resulting powder desiccated until a constant weight was reached. The powdered male worms were then suspended in sterile physiological saline in such a concentration that each 0.25 ml. of suspension represented 21.9 male worms. This concentration was selected because of the information on the recovery of worms obtained from the animals used in Experiment I. An average of 16.7 females and 19.6 males were recovered from the animals in that experiment. The particulate suspension of male worms was refrigerated for 3 days before use.

Two groups of mice harboring unisexual female infections, ranging from 17½ through 24 weeks in duration, received intraperitoneal injections of the particulate suspension of male worms. The mice in Group A received 6 daily injections of 0.25 ml. each representing a total of 131.4 male worms per mouse. The animals in Group A were autopsied at half-week intervals from ½ through 2½ weeks after the last injection. The mice in Group B received 12 daily injections of 0.25 ml. representing a total of 262.8 male worms per mouse. The animals in Group B were autopsied at half-week intervals from 1 through 3 weeks after the last injection.

Two groups of control animals harboring unisexual female infections were used. One group with a female infection 24 weeks old was exposed to male-producing cercariae to determine if the immature female worms of this age were still capable of developing to sexual maturity in the presence of male worms. The second group of control animals did not receive any injections and was not exposed to male cercariae.

TABLE 4.—*Average measurements (mm.) of worms from Experiment IV: Injection of particulate suspension of male worms into mice harboring unisexual females*

Age of exp. worms in weeks	No. wks. after last injection	No. exp. mice used	No. exp. ♀ meas.	Experimental ♀		Experimental ♀ ovary		Age of control worms in weeks	No. control mice used	No. control worms meas.	Control ♀		Control ♀ ovary	
				Length	Width	Length	Width				Length	Width	Length	Width
Group A.—6 injections														
19–26	½	2	23	4.16	0.108	0.120	0.031							
19½	1	3	13	4.37	0.121	0.139	0.030	27	1	2	4.53	0.127	0.247	0.032
20	1½	3	27	4.83	0.117	0.148	0.030							
27	2	2	15	4.03	0.115	0.157	0.037	28	1	10	4.70	0.119	0.191	0.045
27	2½	3	29	3.93	0.104	0.145	0.039							
Group B.—12 injections														
26	½	2	15	3.64	0.138	0.127	0.039							
26	1	2	9	4.14	0.112	0.168	0.036	28	1	10	4.70	0.119	0.191	0.045
28	1½	2	20	3.14	0.104	0.110	0.036							
28	2	2	17	3.90	0.126	0.127	0.051	30	1	22	5.03	0.130	0.213	0.039
29	2½	2	26	4.88	0.105	0.175	0.045							
22–29	3	2	39	4.14	0.113	0.156	0.042	29	2	23	5.10	0.126	0.205	0.043

Microscopic examination of stained toto-mounts of the worms from the experimental animals in Groups A and B revealed no evidence of sexual development of the female worms. All worms were typical sexually immature females. No eggs were found in the tissue press examinations or in the feces of the mice. Measurements (Table 4) made on the worms from animals receiving injections are approximately equal to those of worms from the control animals receiving neither injections of male worm suspension nor exposure to male cercariae. The control animals which were exposed to male-producing cercariae when the unisexual female infection was 24 weeks old were autopsied 7½ weeks after exposure to males. All of the female worms had reached sexual maturity and eggs were easily found in the tissues and feces of these animals. Thus the age of the unisexual female worms could not have been a factor in the failure of the female worms in the experimental animals to develop to maturity in this experiment.

EXPERIMENT V

Addis (1946) demonstrated a relationship between the normal growth of *Hymenolepis diminuta* and the sex hormones, testosterone and progesterone. In

view of this and earlier hypotheses regarding hormonal influence on the development of schistosomes the following experiment was performed to determine whether or not testosterone exerted any influence on the growth or development of the immature unisexual females of *S. mansoni*..

Two groups of mice harboring unisexual female infections ranging from 18 through 33 weeks were used as experimental animals. Mice in Group I received 5 daily intraperitoneal injections of 1 mg. testosterone propionate. The animals were autopsied as follows: same day that the last injection was given; at one-half week intervals from ½ through 2 weeks and 4 weeks after the last injection. Animals in Group II received 10 daily injections of 1 mg. testosterone propionate. The injections in Group II were given over two 5 day periods with 2 days rest between injection periods. The autopsy schedule was the same as for Group I except that the last animals were autopsied 3 weeks after the last injection. Two groups of control mice harboring unisexual female infections were used, one group composed of male mice and the other of female mice.

Although the administration of a total dose of 5 or 10 mg. of testosterone propionate to the experimental animals did not stimulate the unisexual female worms to complete their sexual development, some changes were observed in the worms from the mice receiving testosterone which were not observed in worms from any of the control mice. In Group I, which received 5 mg. of testosterone, the worms from the animals autopsied immediately and ½ week after the last injection showed no evidence of any changes; they were all typical sexually immature females. One week after the end of injections, one worm (4%) out of 25 examined had in addition to the normal ovary a round mass of ovarian tissue 0.032 mm. in diameter, 0.52 mm. anterior to and separate from the regular ovary (Fig. 5). The regular ovary of this worm measured 0.117×0.039 mm. which is within the range of ovarian measurements for this experiment (Table 5). Egg shells containing amorphous material were seen in the uterus of 3 (12%) of the 25 worms examined. There was no evidence of vitelline development in the worms examined and no cellular changes in the ovary.

Twenty-three worms were examined from mice autopsied 1½ weeks after the last injection. Three (13%) worms had ovarian anomalies and 3 (13%) had from 1 to 9 abnormal eggs in the uterus. There was no relationship between the presence of ovarian anomalies and the presence of abnormal eggs. The ovarian anomalies were of three types. One worm had a round mass of ovarian tissue, 0.039 mm. in diameter, 0.017 mm. anterior to and separate from the normal ovary which measured 0.214×0.039 mm. This is essentially the same type of anomaly as that previously described except that the round mass of ovarian tissue was closer to the anterior tip of the ovary. A second worm had two ovaries in tandem (Fig. 4). The anterior ovary measured 0.155×0.036 mm. and appeared to be normal except that it did not have the typical funnel-shaped chamber at the posterior end and this ovary was not connected with an oviduct. The distance between the two ovaries was 0.035 mm. The posterior ovary measured 0.156×0.033 mm. and did possess the posterior funnel-shaped chamber which was connected to the oviduct of the worm. The third worm with an ovarian anomaly had an extremely long ovary (Fig. 6), measuring 0.597×0.039 mm. This ovary is nearly 4 times the average length of the ovary for unisexual females. It possessed a normal funnel-shaped chamber at the posterior end and an apparently mature ovum was seen in the ovi-

duct (Fig. 6). However, there was no evidence of maturation of ova in the ovary of this worm.

Eleven worms were examined from the animals autopsied 2 weeks after the end of injections. No ovarian anomalies were seen and none of the worms had any eggs in the uterus.

Forty-three worms were examined from the animals autopsied 4 weeks after the last injection. Two worms (4%) had ovarian anomalies and 3 worms (7%) had abnormal eggs in the uterus. The ovarian anomalies were in the form of a mass of ovarian tissue anterior to and separate from the normal ovary. One of the masses instead of being round was sub-ovate in shape measuring 0.044 × 0.031 mm. and was 0.029 mm. anterior to the normal ovary. The other mass was round, measuring 0.039 mm. in diameter, and was only 0.002 mm. away from the anterior tip of the normal ovary.

No ovarian anomalies were observed in any of the worms from the experimental animals in Group II, which received 10 mg. of testosterone. One worm from the

TABLE 5.—*Average measurements (mm.) of worms from Experiment V: Injection of testosterone propionate into mice harboring females only*

Age of exp. worms in weeks	Time after injection weeks	No. exp. mice used	No. exp. ♀ meas.	Experimental ♀ Length	Experimental ♀ Width	Experimental ♀ ovary Length	Experimental ♀ ovary Width	Age of control worms in weeks	No. control mice used	No. control ♀ meas.	Control ♀ Length	Control ♀ Width	Control ♀ ovary Length	Control ♀ ovary Width
Group 1—5 mg. testosterone								Control II—♂ mice with ♀ worms						
30	0	2	21	3.32	0.119	0.123	0.036	28½	3	19	4.78	0.122	0.168	0.041
31	½	2	4	4.00	0.202	0.132	0.054							
29–30	1	2	26	5.26	0.092	0.202	0.040							
30	1½	2	24	5.54	0.100	0.219	0.037							
30	2	2	11	4.89	0.110	0.144	0.038							
40	4	2	38	4.96	0.115	0.165	0.034							
Group 2—10 mg. testosterone								Control II—♀ mice with ♀ worms						
22	0	2	13	4.26	0.098	0.110	0.029	22	1	10	4.72	0.115	0.151	0.031
22–33	½	2	49	4.23	0.097	0.116	0.029							
22	1	2	29	4.84	0.111	0.136	0.040	20	1	8	2.25	0.160	0.059	0.019
22	1½	2	5	3.60	0.099	0.112	0.033							
26–30	3	2	24	4.61	0.113	0.140	0.031	28	1	4	4.74	0.109	0.146	0.031

animals autopsied 3 weeks after the end of injections had an abnormal egg in the uterus and one worm had yolk material in the vitelline duct but the vitellaria did not appear to be any different from those seen in the normal sexually immature female.

Worm measurements and ovarian measurements made on the worms from the experimental animals were essentially the same as the measurements made on the worms from the control animals (Table 5). The controls showed that the sex of the host animal had no influence on the development of the worms or in the production of ovarian anomalies. It is interesting that all of the ovarian anomalies observed were in worms from the mice receiving a total of 5 mg. of testosterone propionate. The number of worms showing abnormal eggs in the uterus was also greater in the animals receiving 5 mg. of testosterone.

EXPERIMENT VI

The purpose of this experiment was to determine the effect of living mature male *S. mansoni* on the sexual development of unisexual female worms when the

male worms were implanted in the peritoneal cavity of mice harboring unisexual female infections. The male worms used were recovered from bisexual infections in mice and guinea pigs. Only living undamaged male worms were used. The experimental mice harboring unisexual female infections of 25–34 weeks duration were anaesthetized with intraperitoneal injection of pentobarbital sodium (Nembutal), and, using aseptic technique, a small incision was made in the right lower quadrant. Twenty living male worms were placed in the peritoneal cavity and the incision was closed with silk sutures. These mice were autopsied at weekly intervals from 2 through 8 weeks after the implantation of male worms. Two groups of control mice were used, both harboring unisexual female infections. Group I remained untreated. Group II was exposed to male-producing cercariae to determine if unisexual females 43–45 weeks of age were still capable of developing to sexual maturity in the presence of male worms.

No evidence of sexual development was observed in the female worms from either the experimental animals or the mice in control Group I. Most of the male worms which had been implanted in the peritoneal cavity of the experimental mice

TABLE 6.—*Average measurements (mm.) of worms from Experiment VI: Implantation of living of worms into mice harboring unisexual females*

Age of worms in weeks	Time after imp. ♂ weeks	No. exp. mice used	No. exp. ♀ meas.	Experimental ♀		Experimental ♀ ovary		Age of control worms in weeks	No. control mice used	No. control ♀ meas.	Control ♀		Control ♀ ovary	
				Length	Width	Length	Width				Length	Width	Length	Width
25–28	2	2	13	3.22	0.173	0.099	0.039							
25	3	1	2	2.86	0.134	0.077	0.034							
25	4	2	34	4.13	0.117	0.137	0.030							
26	5	1	18	4.14	0.120	0.140	0.029	47	1	19	5.79	0.123	0.269	0.041
27	6	2	22	4.90	0.126	0.142	0.033							
32	7	1	10	3.44	0.149	0.107	0.044							
29–34	8	2	20	4.48	0.125	0.134	0.033							

were dead at the end of two weeks. A whitish nodule could nearly always be found adhering to the abdominal wall near the site of the incision in which the males were encapsulated. Sections made of these nodules revealed male worms in varying stages of degeneration, and invaded by numerous polymorphonuclear leucocytes. The average length of the female worms and ovaries from the animals in Control I (Table 6), were somewhat greater than those from the experimental animals, but were within the range of lengths of worms and ovaries in unisexual female infections. The animals in Control II were autopsied 9 to 11 weeks after exposure to male-producing cercariae and all of the females were sexually mature. Thus unisexual females 43 to 45 weeks of age are still capable of developing to sexual maturity in the presence of male worms.

DISCUSSION AND CONCLUSIONS

Severinghaus (1928) working with *S. japonicum* showed that when an animal harboring a unisexual female infection was exposed to male-producing cercariae, the sexually immature females completed their sexual development more rapidly than in a normal bisexual infection where male and females are introduced into the animal simultaneously. His most striking example of this was the maturation of 128 day old unisexual females, 19 days after the introduction of males. Faust and

Meleney (1924) stated that in a bisexual infection with *S. japonicum* sexual maturation of the females is not reached until 30 days after exposure.

In our first three experiments it is possible to compare in a broad sense the rate of sexual development of the females, considering such phases of maturation as: the first appearance of worms in copula; first observation of spermatozoa in the fertilization chamber of the females; first observation of a normal egg in the oötype; first evidence of the functioning of the vitellaria; first appearance of eggs in the tissues and in the feces of the animals. Table 7 summarizes these observations for Experiments I, II and III. In Experiment I males were added to an existing female infection, in Experiment II females were added to an existing male infection and in Experiment III males and females were introduced at the same time. In all three experiments all of the female worms were found to be mature in 7 weeks. Although the above observations, summarized in Table 7, are based on only 1 or 2 animals in each instance and although there are natural variations between animals as well as between worms, it is reasonable to conclude that the rate of sexual maturation of *S. mansoni* females is not accelerated either by the female's having reached a fairly advanced stage of development prior to the introduction of male worms or by introducing the female worms after the male worms have reached sexual maturity.

TABLE 7.—*Comparison of the rate of sexual development of the female worms in Experiments I, II and III*

	Experiment I Males added to existing Female infection	Experiment II Females added to existing Male infection	Experiment III Males & Females introduced simultaneously
Worms first found in copula	4½ weeks	4 4/7 weeks	5 weeks
Spermatozoa first seen in Female ..	5½ "	5 2/7 "	5 4/7 "
Normal egg first seen in Female ..	5 "	5 4/7 "	5 4/7 "
Vitellaria first functional	5 "	5 2/7 "	5 4/7 "
Eggs first seen in tissues.....	5 "	6 4/7 "	5 4/7 "
Eggs first seen in feces	6 "	7 2/7 "	7 "

Vogel (1941) concluded that the nature of the influence exerted by one sex of schistosome on the other is not known, nor is the manner in which it is brought about. He discussed two hypotheses. One assumes the presence of a hormonal substance which is transferred directly from male to female either with the sex products or through the integument of worms. Our observations on the development of the males and females would lend support to the hypothesis that a hormonal substance may be transferred to the female worm with the spermatozoa. Of course it is possible that the spermatozoa themselves provide the stimulus for sexual maturation of the females. In Experiments I and III we saw no evidence of sexual maturation of the female worms until spermatozoa were seen in the seminal vesicle of the male worms. The presence of spermatids alone did not seem to stimulate any sexual development of the females. In Experiment II, where the male worms were sexually mature with spermatozoa in the seminal vesicle when the females were introduced, the first evidence of maturation of the females was coincidental with the appearance of spermatozoa in the fertilization chamber of the female.

The second hypothesis discussed by Vogel is based on the observation that the sexually immature females seldom contain black hematin in the intestinal ceca as is commonly found in sexually mature paired worms, and assumes that the females with weak muscles can not feed normally unless paired with the stronger males. Our observation that the intestinal ceca of the unisexual female *S. mansoni* usually

contain bright red undigested blood is in agreement with Vogel's observation and might lend support to this hypothesis. However, as Vogel stated, it is more difficult to explain the influence of the female *S. japonicum* on the growth of the male by this hypothesis.

We were unable to produce sexual maturation of female *S. mansoni* except by affording them the opportunity of residence in the gynecophoral canal of the male worm in a normal manner. The production of ovarian anomalies and the increase in the formation of abnormal eggs in unisexual females from mice receiving 5 mg. of testosterone propionate might be an indication that the influence of the male on the female is hormonal in nature. In spite of the fact that none of the worms from the mice which received 10 mg. of testosterone showed ovarian anomalies or an increase of development of abnormal eggs, it is felt that this mammalian male hormone did exert some influence on the reproductive cells of the female worms and it is probable that a schistosome male hormone exists which provides the stimulation for the maturation of the female worm. Our previous observation (Moore, *et al.* 1949b) that, in unbalanced bisexual infections in which there is a great preponderance of female worms, the unmated females are almost always retarded in sexual development similarly to those in unisexual female infections, lends support to our conclusion that the principal, if not the only, stimulus to sexual maturation of the female *S. mansoni,* is provided by the products of the male worm in the act of copulation.

SUMMARY

This series of experiments shows that living mature male *S. mansoni* in the mesenteric portal system of mice and hamsters are essential for the sexual maturation of female worms. The female worms are not stimulated to mature until the male worms have reached sexual maturity. The rate of growth and sexual maturation of the female worms is not significantly altered by the introduction of males into an animal harboring a unisexual female infection of 11 weeks duration, or by the introduction of female worms into an animal harboring a unisexual male infection in which the males are sexually mature, as compared with the introduction of males and females simultaneously.

Attempts were made to produce sexual maturation of unisexual female *S. mansoni* by (1) giving the host animal intraperitoneal injections of a particulate suspension of desiccated mature male worms, (2) giving the host animal injections of testosterone propionate, and (3) implanting living mature male worms in the peritoneal cavity of mice harboring unisexual female worms. Sexual maturation of the female worms was not completed in any of these experiments. Ovarian anomalies such as the formation of an extra mass of ovarian tissue, an abnormally long ovary and an increase in the formation of abnormal eggs were observed in some of the worms from mice receiving 5 mg. of testosterone propionate. Female worms 315 days old in unisexual infections were found to be still capable of completing their sexual development and maturation when fertilized by male worms.

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Explanation of Plates

All drawings made with the aid of a camera lucida from stained toto-mounts of the worms.

Abbreviations Used

F—funnel-shaped chamber of the ovary; FC—fertilization chamber of the oviduct; O—ovary; OT—oötype; OV—ovum.

Plate I

Fig. 1. Sexually immature female *S. mansoni* 13 weeks old from a unisexual infection, control mouse Experiment I.

Fig. 2. Ovary of sexually immature *S. mansoni* from a unisexual infection, control mouse Experiment II.

Fig. 3. Anterior portion of sexually mature female *S. mansoni* 9 weeks old from a bisexual infection.

Plate II

Ovarian anomalies seen in immature female *S. mansoni* from unisexual infection in mice which received a total dose of 5 mg. testosterone propionate. Worms 31½ weeks old, recovered 1½ weeks after end of injections.

Fig. 4. Double ovary.

Fig. 5. Round mass of ovarian tissue anterior to and separate from normal ovary.

Fig. 6. Abnormally long ovary. Note ovum in oviduct.

PLATE I

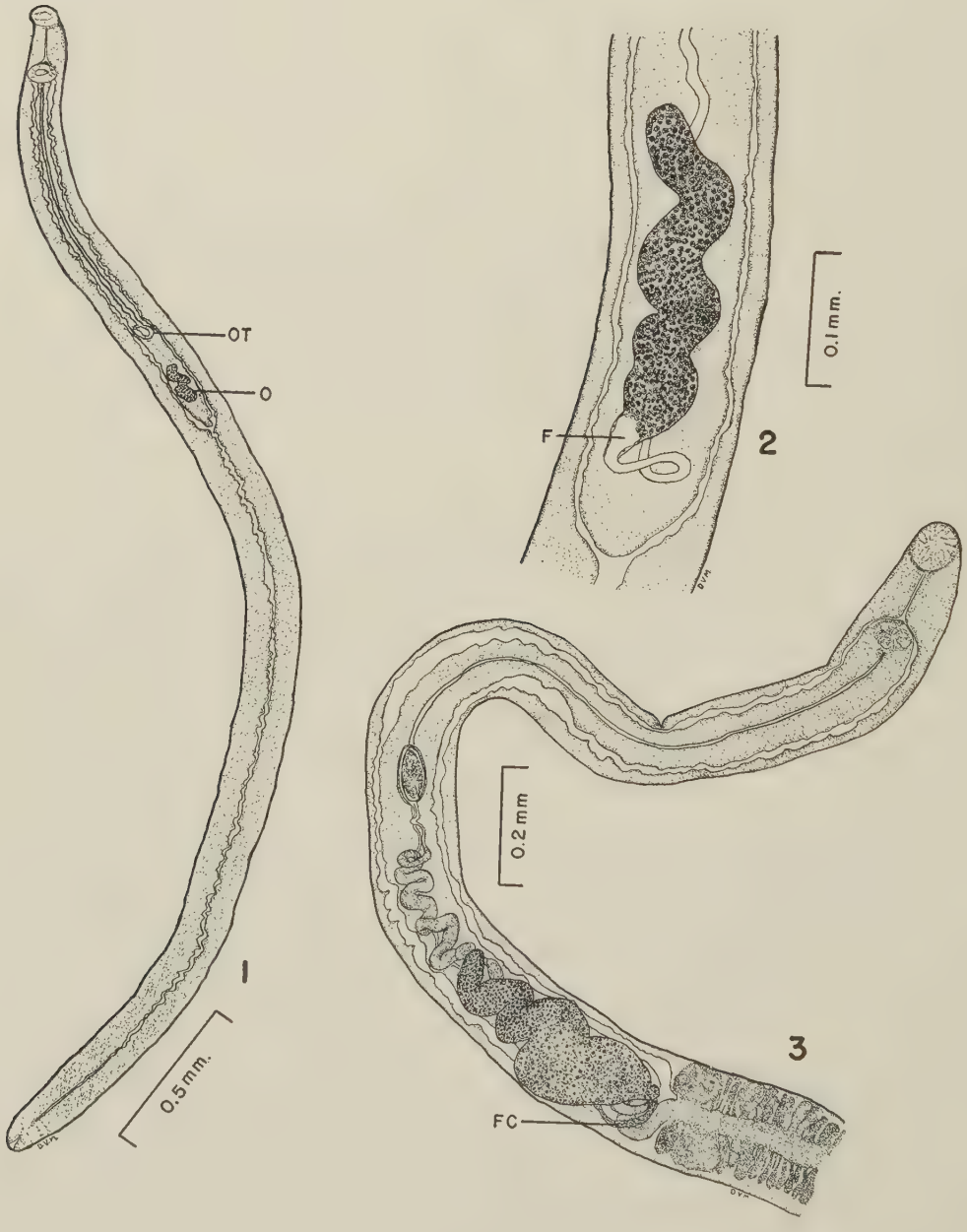
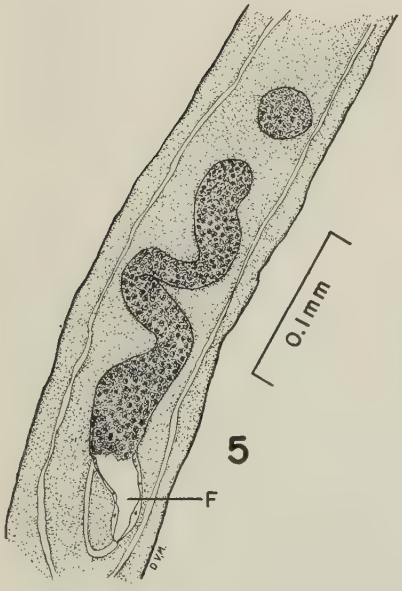
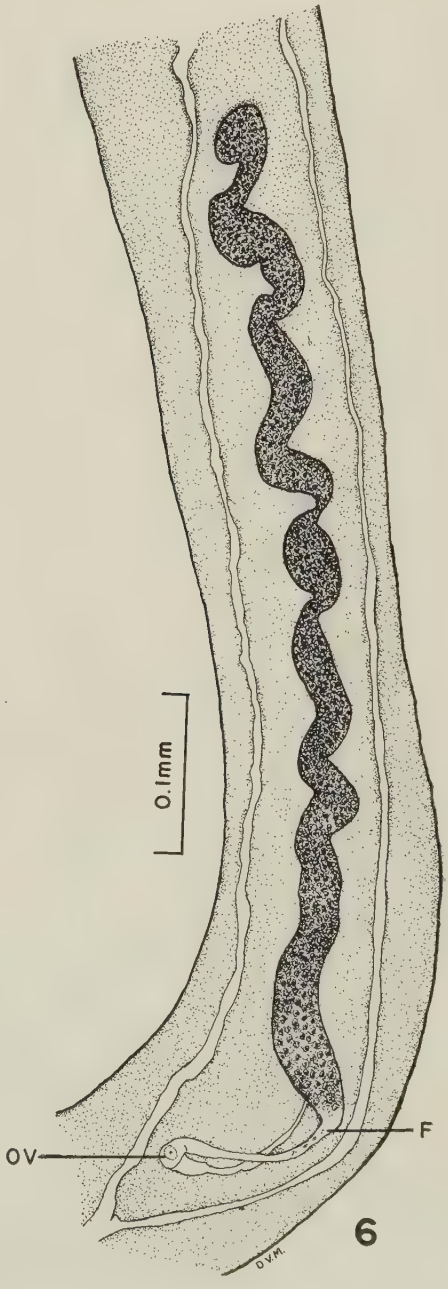


PLATE II





MYTILICOLA PORRECTA N. SP. (COPEPODA: CYCLOPOIDA) FROM THE INTESTINE OF MARINE PELECYPODS[1]

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A new genus and species of cyclopoid copepod, *Mytilicola intestinalis*, from the intestine of a mussel, *Mytilus galloprovincialis* Lamarck, in the Gulf of Trieste, was described by Steuer (1902). This was followed by a more detailed description including the internal anatomy (Steuer, 1903). Certain postembryonic stages have been studied by Pesta (1907) and Caspers (1939), and cytological details of the adults have been described by Ahrens (1937, 1939a, b, and c). Biological aspects of this species have been investigated by Odlaug (1946), Baird, Bolster, and Cole (1951), Grainger (1951), and Cole and Savage (1951). Further information on the biology of this copepod is to be found in the papers by Korringa, Korringa and Lambert, Heldt, Lambert a and b, Leloup, Cole, Meyer and Mann, Havinga, and Dollfus published in 1951 as results of the conference on *M. intestinalis* in Paris.

Trochicola enterica Dollfus 1914, found in the intestine of marine snails along the coast of France, should probably be considered identical with *M. intestinalis,* as suggested by Monod and Dollfus (1932). A single specimen found by Pearse and Wharton (1938) in an oyster in Apalachicola Bay on the west coast of Florida was identified as *M. intestinalis* by C. B. Wilson, but probably belongs instead to the new species described below. Dr. Pearse has reported in correspondence that the whereabouts of this specimen is not now known.

Except for the doubtful Florida specimen, *M. intestinalis* is known only from Europe, where it has been reported from the Adriatic Sea (Steuer, 1902 and 1903, Pesta, 1907, Ahrens 1939a), from the Mediterranean Sea (Vayssière, 1914, Dollfus, 1927, Cerruti, 1932, Ahrens, 1939a, Bassedas, 1950), from Germany (Caspers, 1939), from the northern coast of France (Dollfus, 1914 and 1927, Monod and Dollfus, 1932), from England (Ellenby, 1947, Grainger, 1951, Cole and Savage, 1951), and from Ireland (Grainger, 1951). Other distribution records are to be found in the papers of the conference on *M. intestinalis* already referred to.

The known natural hosts are *Mytilus galloprovincialis* Lamarck, *Mytilus edulis* Linné, *Ostrea edulis* Linné, *Cardium edule* Linné, and (if the synonymy of *Mytilicola* and *Trochicola* is admitted) three gastropods, *Zizyphinus zizyphinus* Linné, *Gibbula cineraria* Linné, and *Gibbula varia* Linné. Artificial infections have been obtained by Hepper (1953) in *Mytilus edulis* Linné, *Ostrea edulis* Linné, *Cardium edule* Linné, *Crepidula fornicata* Linné, and *Paphia pullastra* (Montagu).

A second species, *Mytilicola orientalis,* was described by Mori (1935) from the digestive tract of *Ostrea gigas* Thunberg and *Mytilus crassitesta* Lischke in the Inland Sea of Japan. Apparently unaware of the work of Mori, C. B. Wilson (1938) described *Mytilicola ostreae* from *Ostrea gigas* imported from Japan into Puget Sound. As pointed out by Odlaug (1946), this species is probably identical with *M. orientalis,* but a final decision will depend upon a comparison with Japa-

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[1] The material upon which the new species herein described is based was collected while the author was a guest investigator at the Louisiana State University Marine Laboratory at Grand Isle, Louisiana.

nese specimens. According to Odlaug (1946), the species is widespread in lower Puget Sound, where it occurs most often in *Mytilus edulis* Linné, but also in *Ostrea lurida* Carpenter, *Ostrea gigas* Thunberg, *Paphia staminea* Conrad, and the gastropod, *Crepidula fornicata* Linné.

Two other species, both assigned to the genus *Piratasta* Leigh-Sharpe 1934, may actually belong instead to *Mytilicola*. *Piratasta virgatula* Leigh-Sharpe 1934, found in *Laternula* (*Anatina*) sp. at the Siboga Expedition Station 50 on the west coast of Flores, and *Piratasta brachidontis* Yamaguti 1939, found in *Brachidontes senhausi* (Reeve) from Lake Hamana, Siquoka Prefecture, Japan, seem to resemble *Mytilicola* very closely. No generic diagnosis of *Piratasta* was given by Leigh-Sharpe (1934) and no comparison made with *Mytilicola*. The descriptions by Yamaguti (1939) and by Leigh-Sharpe (1934) are so wanting in details that it is impossible to determine many points. In both cases males are unknown.

During June, 1947, both sexes of a new species of *Mytilicola* were collected from the intestine of the ribbed mussel, *Modiolus demissus granosissimus* Sowerby, the recurved mussel, *Mytilus recurvus* Rafinesque, and the hard-shelled clam or quahaug, *Venus mercenaria mercenaria* Linné, in the marshes and bayous behind Grand Isle and Grand Terre, Barataria Bay, Louisiana.

I wish to thank Dr. John S. Rankin for study specimens of *M. orientalis* from Puget Sound, Mr. J. N. R. Grainger for *M. intestinalis* from Ireland, Dr. H. A. Cole and Mr. B. T. Hepper for the same species from England, and Dr. Fenner A. Chace Jr. for the loan of specimens from the United States National Museum.

Mytilicola porrecta n. sp.

Female: In living specimens the body is reddish to brownish orange and the egg sacs are white to greenish blue, depending upon the age of the embryos. The total length, based on an average of five specimens, is 4.875 mm. (4.017–5.850 mm.). At the anterior border of the somite bearing the second pair of legs the body width is 0.377 mm. (0.343–0.414 mm.). The five pairs of dorsolateral thoracic projections are nearly of equal length (Figs. 1, 2, and 3). The abdomen is slender and elongated, terminating in two small caudal rami which extend posteriorly and bear four small spines (Fig. 4). There is no distinct cuticular division denoting thoracic or abdominal somites, but the segmentation is suggested by the internal muscle arrangement. The egg sacs, 1.28×0.15 mm., and containing about 45–55 eggs, extend well beyond the ends of the caudal rami.

The first antenna (Fig. 5) has four podomeres, the three distal ones each armed with an aesthete and several setae or spines, the proximal one large and armed with scattered spines and a group of very fine spinules along its anterior border. The second antenna (Fig. 6) appears also to have four podomeres, the last being a strongly chitinized hook curving slightly toward the midline of the body. The mandibles are absent. The first maxilla (Fig. 7) consists of a circular base from which two setae arise. The second maxilla (Fig. 8) appears to have three podomeres, the basal one elongated and by far the largest, the middle one short, and the distal one spatulate and fringed with a row of slender setae. The maxillipeds are lacking, although their position may be indicated by two chitinous thickenings in the cuticula.

The relative positions of the mouth parts may be seen in a ventral view of the

head (Fig. 9). A medially notched upper lip lies in front of and somewhat ventral to the mouth opening. The lower lip is a transversely elliptical flap. On the ventral surface of both sides of the head just behind the bases of the second antennae there are two small papilla-like structures and adjacent areas of fine cuticular markings. Internally there is a strongly chitinized framework composed of several pieces. The head measures 0.392 mm. in length and 0.418 mm. in width, the ratio of length to width being 0.924.

The first swimming leg (Fig. 10), like the remaining three, is biramous. Both rami have two podomeres, expanded on their outer margins to form a wide lamella. The first exopodite podomere bears a single seta on its outer distal corner, and the second bears three terminal setae. The endopodite is unarmed. The second leg is like the first, except for the reduction in size of the outermost terminal exopodite seta (Fig. 11). The third and fourth legs have only two such terminal setae (Fig. 12). In the coxopodite and basipodite region of all four pairs of legs there are two long, curved, chitinous bars which form part of the whole framework upon which the leg rests (Fig. 13). (The two bars appear straighter in the drawings because of dissection and pressure of the coverglass.) The fifth leg is absent. The openings of the oviducts are strongly chitinized and show a transverse rod across the aperture (Fig. 14).

Male: The color is reddish to brownish orange, but paler than in the female. The total length, based on five individuals, is 2.472 mm. (2.379–2.574 mm.), and the width measured as in the female is 0.319 mm. (0.272–0.340 mm.) The dorsolateral thoracic projections are reduced to almost undiscernible humps (Fig. 15). These are visible only when light is reflected from a specimen partially submerged in clearing oil, and not seen in ordinarily mounted specimens. The abdomen (Fig. 16) is elongated, without distinct segmentation, and terminates in caudal rami like those of the female.

The first and second antennae and the first and second maxillae are like those of the female. The mandibles are absent. The maxilliped is well-developed, and consists of a large, somewhat triangular basal podomere and a terminal podomere in the shape of a well-chitinized, strongly recurved hook (Fig. 17). There is a hyaline lamella along the concave margin of the hook. The relative positions of the mouth parts are indicated in Fig. 18.

The four swimming legs resemble those of the female except for their setal armature. The endopodites of the legs (Figs. 19, 20, and 21) bear long terminal setae, which are easily broken off in preserved specimens and thus may appear to be absent. The second and third legs are similar. One specimen was observed in which the armature of the legs was not typical, as indicated in Figs. 22, 23, 24, and 25. The fifth leg is an erect lobe with three terminal setae and one on the outer margin (Figs. 26 and 27). The outer marginal seta may belong to the basal podomere, since there is an indistinct subdivision of the leg. The sixth leg is probably represented by a flat, rounded lobe, bearing two setae, located on the posterior area of the ventral surface of the sixth thoracic somite (Figs. 16 and 28).

The holotype Cat. No. 95741, allotype Cat. No. 95743, and sixty paratypes Cat. No. 95743 have been deposited in the United States National Museum, twenty paratypes in the British Museum (Natural History), and the remaining paratypes in the author's collection.

HOST RELATIONSHIPS

Mytilicola porrecta was found in pelecypods inhabiting tidal marshes and bayous along the Barataria Bay sides of Grand Isle and Grand Terre. The salinity of the water in the area was about 25 grams per liter. The copepods occurred most commonly in *Modiolus demissus granosissimus* Sowerby, with 101 of 241 or 42 per cent parasitized. The next most common host was *Mytilus recurvus* Rafinesque, with four of 22 or 18 per cent parasitized. (Another group of 23 from deeper water, not exposed by the tide, was unparasitized.) One *Venus mercenaria mercenaria* Linné from the marsh contained a single specimen of *M. porrecta.* A total of 252 *Ostrea virginica* Gmelin from pools in the tidal marsh was examined, but all were negative, even though they had been living side by side with the parasitized *Modiolus.* One hundred and forty-eight oysters from commercial oyster beds, in water deep enough to remain covered during the lowest tides, were also negative.

The greatest number of copepods found in a single *Modiolus* was 15 (five males and ten females), the average number per parasitized *Modiolus* being 2.75.

DIFFERENTIATION OF SPECIES OF *Mytilicola*

The following table points out among the three species of *Mytilicola* certain differences which may be easily observed in suitable whole mounts without dissection.

TABLE 1.—*Differentiation of the three species of the genus* Mytilicola

	M. intestinalis Steuer	*M. orientalis* Mori (= *M. ostreae* Wilson)	*M. porrecta* n. sp.
FEMALE			
caudal ramus	elongated (237μ) and widely divergent (Fig. 29)	elongated (233μ) but not widely divergent	short (96μ) and not divergent (Fig. 4)
posterior corners of head	smoothly rounded (Fig. 29)	with a pair of slender processes (Fig. 31)	smoothly rounded (Fig. 1)
dorsolateral thoracic projections	short and rather stout (Fig. 29)	long and tapering (Figs. 31 and 32)	short and rather stout (Figs. 1 and 2)
ratio of length of head to width	0.647	0.782	0.924
egg sac	long, reaching far beyond caudal rami	long, 2.74 mm., reaching far beyond caudal rami (Fig. 32)	short, 1.28 mm., reaching only short distance beyond caudal rami (Fig. 3)
MALE			
dorsolateral thoracic projections	well-formed	well-formed but absent on 1st leg-bearing somite according to Mori (1935)	reduced to almost undiscernible humps (Fig. 15)
claw of maxilliped	elongated, not strongly hooked (Fig. 30)	elongated, not strongly bent (Fig. 33)	short, stout, strongly hooked (Fig. 17)

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EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida. Scale A applies to Figs. 1–3, 16, 29, 31, and 32; scale B to 4–6, 10–14, 19–25, and 28; scale C to 7, 8, 17, 26, and 27; scale D to 9 and 18; scale E to 15; and scale F to 30 and 33.

PLATE I

Mytiliticola porrecta n. sp., female

FIG. 1. Dorsal.
FIG. 2. Lateral.
FIG. 3. Ventral.
FIG. 4. Caudal rami, slightly flattened by pressure.
FIG. 5. First antenna, ventral.
FIG. 6. Second antenna, ventral.
FIG. 7. First maxilla, ventral.
FIG. 8. Second maxilla, middle and distal podomeres only, ventral.
FIG. 9. Head, with second antennae bent forward, ventral. The dotted circle indicates the position of the mouth.
FIG. 10. First leg, anterior aspect.
FIG. 11. Second leg, distal podomere of exopodite only.
FIG. 12. Third leg, distal podomere of exopodite only.
FIG. 13. Chitinous framework at base of second leg, drawn from undissected specimen.

PLATE II

Mytilicola porrecta n. sp.

FIG. 14. Female, chitinous framework around opening of oviduct.
FIG. 15. Male, head and thorax, dorsal.
FIG. 16. Male, ventral.
FIG. 17. Male, maxilliped, ventral.
FIG. 18. Male, head, ventral.
FIG. 19. Male, first leg.
FIG. 20. Male, second leg.
FIG. 21. Male, fourth leg.
FIG. 22. Male, atypical first leg.

PLATE III

Mytilicola porrecta n. sp.

FIG. 23. Male, atypical second leg.
FIG. 24. Male, atypical third leg.
FIG. 25. Male, atypical fourth leg.
FIG. 26. Male, fifth leg, anterior aspect.
FIG. 27. Male, fifth leg, ventral.
FIG. 28. Male, sixth leg.

Mytilicola intestinalis Steuer

FIG. 29. Female, dorsal.
FIG. 30. Male, maxilliped.

Mytilicola orientalis Mori

FIG. 31. Female, head and thorax, dorsal. (Specimen somewhat shrunken in mounting so that edges of thoracic legs show in this view.)
FIG. 32. Female, lateral.
FIG. 33. Male, maxilliped.

PLATE I

PLATE II

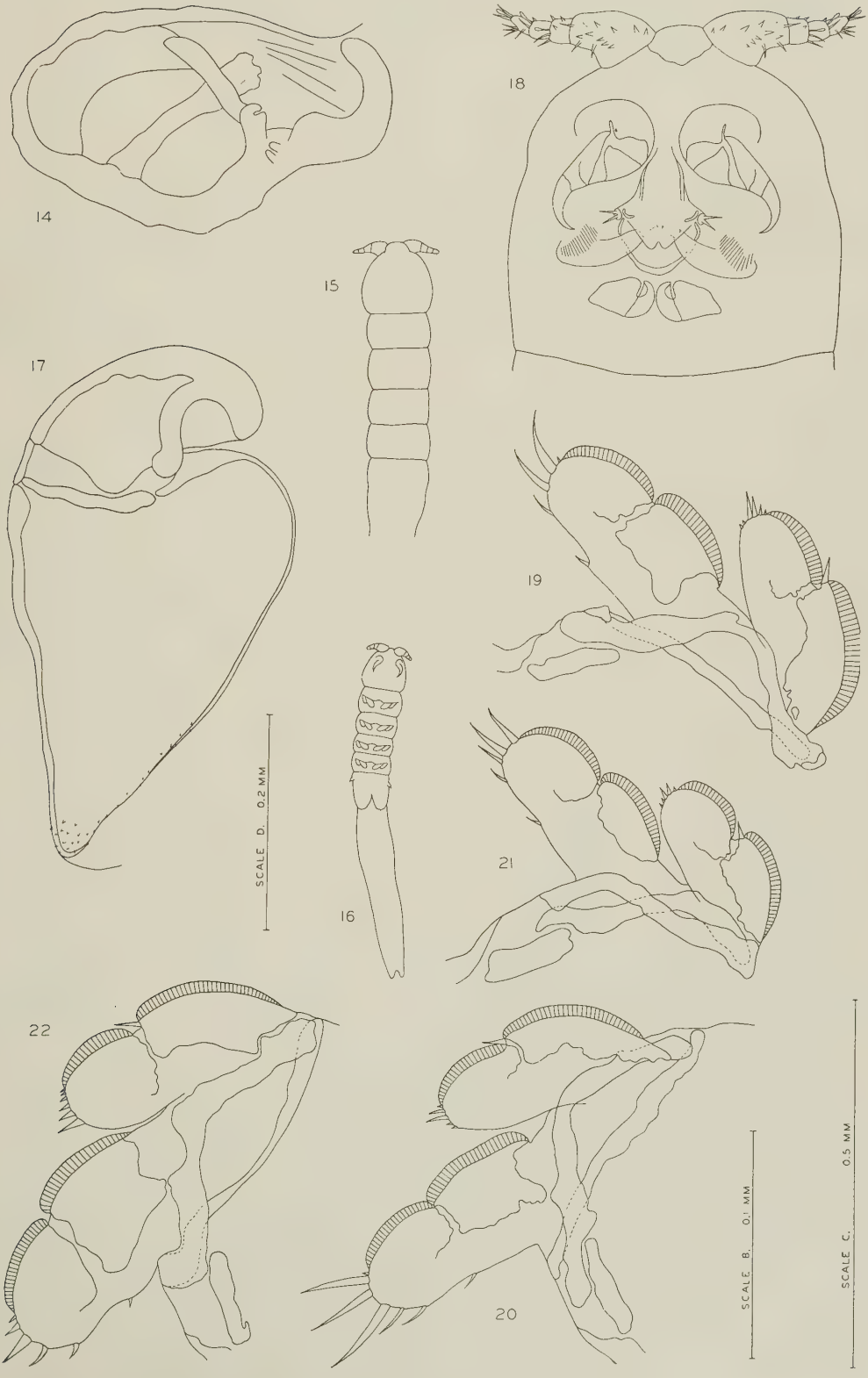
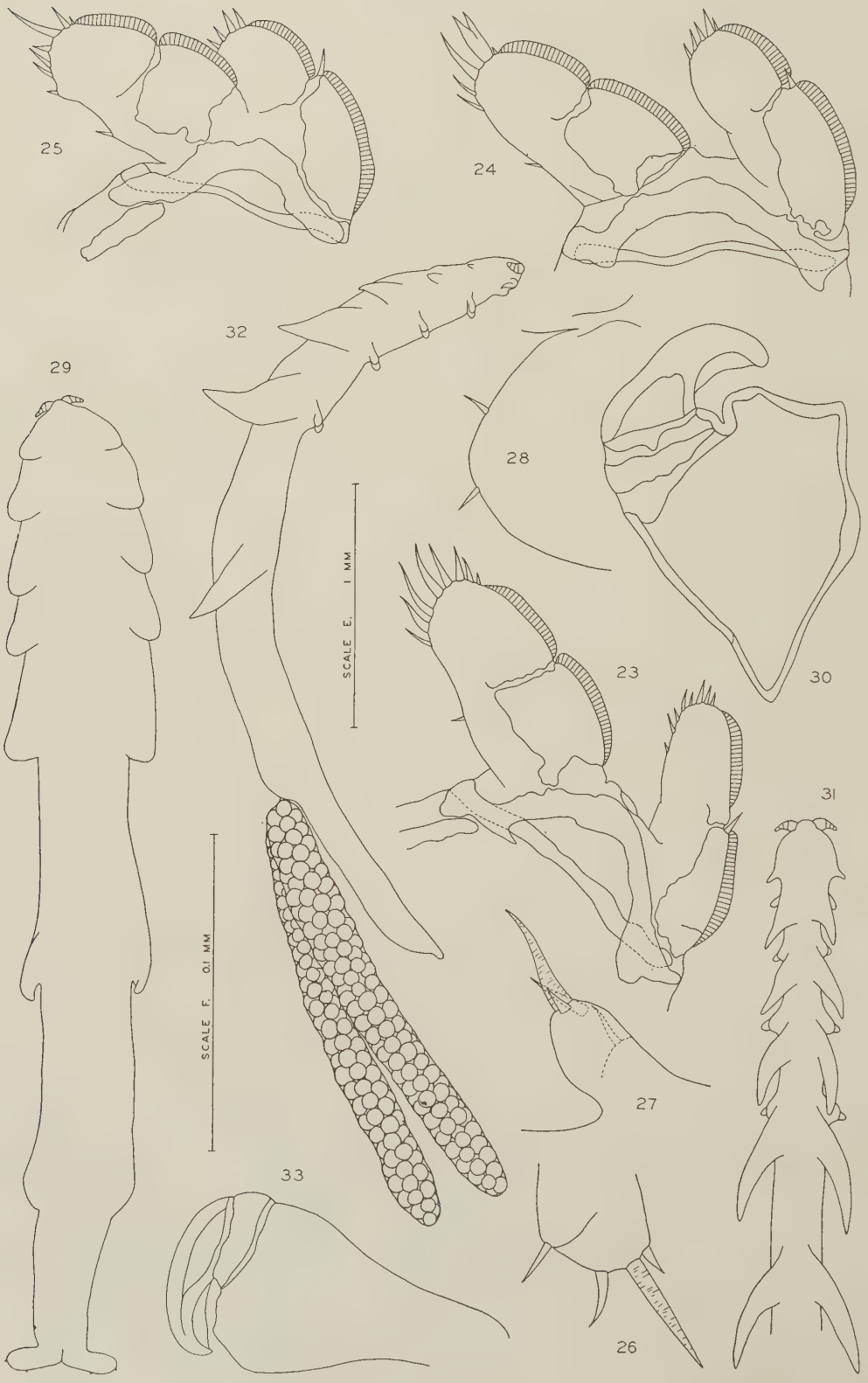


PLATE III



INCIDENCE OF TRYPANOSOMES IN THE CANADA GOOSE AS REVEALED BY BONE MARROW CULTURE

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INTRODUCTION

Herman and Diamond (1952) reported briefly on a method of culturing trypanosomes from avian bone marrow collected at autopsy. Using this technique they demonstrated for the first time the presence of trypanosomes in the Canada goose (*Branta canadensis* L.). Subsequent work has led to a new technique for obtaining bone marrow samples from living birds. This paper gives the details for culturing avian trypanosomes from bone marrow and presents the results obtained in trypanosome surveys of wild Canada geese.

MATERIALS AND METHODS

The first bone marrow samples of this study were obtained from the tibiotarsus (erroneously called the femur in the paper of Herman and Diamond, 1952) of freshly killed birds. As soon after death as possible the lower portion of a leg was severed with a sharp cartilage knife at the articulation of the tibiotarsus and femur. Feathers, skin and muscle were removed from the tibiotarsus following which the bone was dipped in 70% ethyl alcohol and flamed. The proximal head of the bone was then removed with heavy poultry shears which had been sterilized for several hours in alcohol and flamed immediately before use. After dipping the cut end of the bone shaft in alcohol and flaming it, a sterile 15-gauge B-D needle attached to a 10-ml. syringe was inserted into the marrow cavity and a few drops of its contents aspirated. From 0.1 to 0.2 ml. of marrow was found sufficient to inoculate a tube of medium.

Since the above method necessitated killing the bird, thus limiting the applicability, a technique was developed for obtaining the marrow from living birds. This procedure required two individuals, one to hold the bird and the other to obtain the marrow sample. For left leg operation (our preference) the assistant held the goose as in Figure 1. The bird was placed on its back with feet directed toward assistant, and neck bent backward to assistant's left side. The head, right wing and right leg were held by the assistant's left hand, the left wing was folded beneath the body, and the left leg was secured by the right hand. With a slight twisting motion of the assistant's wrist, the left leg was externally rotated and pushed forward so that the head of the tibiotarsus faced the operator. In this position, the assistant could easily restrain movements of the bird permitting the operator to obtain the marrow without interference. A hand towel, soaked in alcohol, and wrapped around the bird provided an aseptic field, kept the breast feathers out of the way and also aided in securing the left wing.

To prepare the site for removal of bone marrow, the proximal head of the tibiotarsus was defeathered and sterilized with alcohol. The prominent inner anterior

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crest of the bone was then located (Figure 2) by palpation. A B-D Lundy-Irving needle (#16-ga. 3″), fitted with a stylet, was introduced with steady firm pressure through the head of the bone into the marrow cavity, entering at a point 0.5 cm. below and slightly to the right of the crest. The needle when in place lies in a plane parallel to the long axis of the bone. The stylet was then withdrawn and a

Fig. 1. Position of goose for biopsy procedures.

10-ml. hypodermic syringe fitted in its place. A steady even pull on the syringe plunger aspirated the bone marrow. After the marrow had been obtained, the needle was quickly withdrawn, the puncture wound bathed in alcohol and the bird released.

Correctly carried out, this procedure does not seriously incapacitate the bird.

Before refinements in procedure were developed, slight lameness (which completely disappeared within 24 hours) was observed in a few of the first birds subjected to this operation. At Pea Island National Wildlife Refuge, 59 Canada geese were subjected to this bone marrow sampling procedure and every bird upon release walked away without lameness and took flight in a normal way. No lame birds were sighted within the next few days. This was considered a reliable observation since the topography of the refuge made it particularly easy to sight sick or injured geese.

The technique has proved satisfactory under both laboratory and field conditions and with slight modification can be used to obtain bone marrow samples from any size bird. Hand pressure exerted on the biopsy needle is adequate to penetrate the tibiotarsus bone in the Canada goose, however, this is not sufficient for the domestic goose and duck which have a rather thick layer of bone in the head of the tibiotarsus. To enter the marrow cavity of these birds it is necessary to drive the needle through the bone with two or three sharp blows of an instrument such as a neurological per-

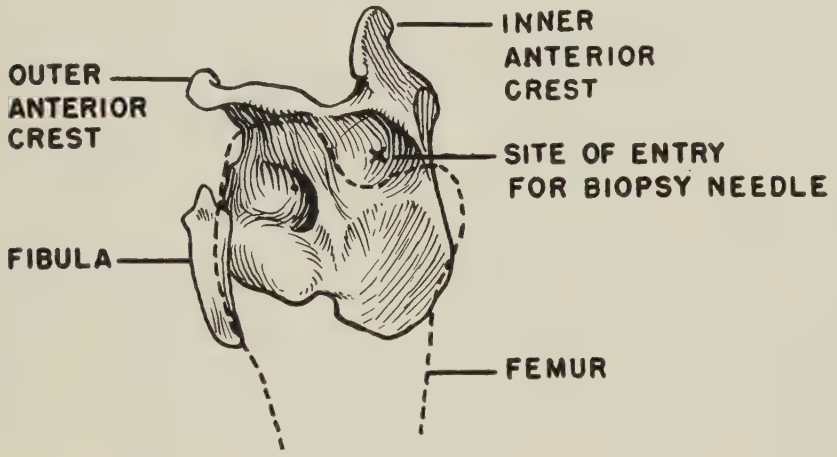

FIG. 2. Surface view of proximal head of left tibiotarsus.

cussion hammer. As might be expected, a greater degree of trauma results from this procedure but the animal soon recovers.

Since most previous reports of trypanosomes from birds have been based on the examination of blood smears, heart blood was cultured in our initial survey of wild geese. This blood was obtained by sterile heart puncture from birds freshly killed and undergoing autopsy.

Two media were used for culturing the bone marrow and heart blood. One of these, Ponselle's (1923) hypotonic broth, WB (water-blood) a monophasic medium, was made, according to the author's direction, by mixing equal volumes of sterile defibrinated rabbit blood and sterile distilled water, under aseptic conditions. The blood employed was not inactivated by heating as in the original Ponselle's medium. The medium was distributed in quantities of 3 ml. to small culture tubes (13×100 mm.), plugged with rubber stoppers, and incubated for 48 hours to check sterility .

The other, a diphasic medium, SNB-9 (saline-neopeptone-blood), was prepared as follows: A broth to be used in the overlay and agar base was made by dissolving

0.6 gm. sodium chloride and 2.0 gm. Difco neopeptone in 100 ml. of distilled water. To prepare the agar base, 2 gm. of agar were added to 100 ml. of broth and dissolved by heating. After autoclaving at 16 lbs., 121° C., 20 min., and cooling to 50° C., 25 ml. of sterile defibrinated rabbit blood were added. The mixture was then tubed aseptically in 2.0 ml. amounts and the tubes slanted to form an agar surface 2.0 cm. in length. Size of the tubes was the same as used for Ponselle's medium. Upon solidification of the agar, 0.5 ml. of sterile broth was added as an overlay to each slant. Rubber stoppers were used to plug the tubes. After a sterility test incubation of 48 hours, the medium was stored at ice box temperature (4° C.) until used. The buffer systems present in rabbit blood maintain the pH of the medium at a fairly constant level. The liquid overlay has a pH of 7.3 after 48 hours' contact with the blood agar base during the period of the sterility check. Twenty-one days after inoculation and incubation with trypanosomes the average pH of several cultures examined was found to be 7.1

Inoculated cultures were incubated at room temperature (21–23° C.). The cultures from the first of the four surveys were examined at 14 and 21 days. However, since 32 of the 33 positive cultures in the above survey were detected on the 14th day of incubation, examinations of cultures in the other three surveys were limited to the 14th day. Examinations were made by removing a loopful of fluid from each tube and studying it under the microscope at 120× and 230×.

Smears made from bone marrow and heart blood were stained with Giemsa, using the following modification of Ponselle's (1913) technique. Smears made in the usual manner were air dried and then fixed for 5 minutes in a solution consisting of 2–4 drops tincture of Iodine USP per 10 ml. of methyl alcohol. They were then rinsed in methyl alcohol and again air dried. To stain, the smears were immersed from 30 to 60 minutes in Giemsa solution prepared by adding 1 drop of Giemsa[2] to 1 ml. of distilled water. After staining they were washed for 30 seconds in distilled water and dried.

During the period 1951–53, four surveys were conducted to determine the incidence of trypanosomes in Canada geese.

Survey I.—Pea Island National Wildlife Refuge, North Carolina. In December 1950, 100 Canada geese were trapped at the refuge and set aside for a study of the relationship of protein level in the diet to parasitism and winter die-off. The geese were caged in outdoor pens and fed controlled diets. On April 15, 1951, the experiment was terminated and 91 geese were brought to Laurel, Md. where they were sacrificed and subjected to complete autopsy. As each bird was killed, bone marrow and heart blood samples were obtained by the methods described above and used to inoculate media as follows: 1 tube each of WB medium with bone marrow and heart blood and 1 tube each of SNB-9 medium with bone marrow and heart blood. Smears were made of bone marrow and heart blood from each of the geese.

The series of 96 geese discussed in the earlier paper of Herman and Diamond (1952) included the above 91 birds plus 5 additional specimens which were used as a preliminary test group.

Survey II.—Pea Island National Wildlife Refuge, North Carolina. On Febru-

[2] Giemsa Stain Solution—a stock solution prepared commercially by National Aniline Division, Allied Chemical and Dye Corporation.

ary 17, 1953 an aspirated bone marrow sample for culture was obtained from each of fifty-nine Canada geese trapped at the refuge during the week of February 8, 1953. Two tubes of SNB-9 medium were inoculated per bird. Smears were not made. Upon completion of the sampling operation, each bird was banded and released.

Survey III.—Blackwater National Wildlife Refuge, Maryland. Forty-eight Canada geese trapped at the refuge and caged temporarily in outdoor pens were subjected to bone marrow sampling on February 26, 1953. Two tubes of SNB-9 medium were inoculated per goose. No smears were made. After completion of the operation each bird was returned to its pen.

Survey IV.—Ninety-eight Canada geese obtained from a number of national wildlife refuges during the months of November 1952 to March 1953 (Table II) were subjected to bone marrow sampling upon arrival at the laboratory. Two tubes of SNB-9 were inoculated per goose. Heart blood smears were made.

RESULTS

Results of the four surveys are presented in Tables I and II. Diagnoses of

TABLE 1.—*Incidence of trypanosomes in Canada geese as revealed in cultures of bone marrow of the tibiotarsus and heart blood obtained at autopsy. Survey I. Pea Island National Wildlife Refuge, North Carolina*

Medium	Source of Culture	No. Geese Examined	Birds from which Culture Was Not Contaminated		Birds from which Noncontaminated Culture Was Positive for Trypanosomes	
			No.	%	*No.*	%
WB	Bone marrow	91	86	94.5	0	—
WB	Heart blood	91	82	90.1	0	—
SNB-9	Bone marrow	91	82	90.1	33	40.2
SNB-9	Heart blood	91	77	84.6	3[1]	3.9

[1] Bone marrow cultures of two of these were also positive, the third was contaminated.

TABLE 2.—*Incidence of trypanosomes in Canada geese as revealed in cultures of bone marrow from the tibiotarsus of living birds. Surveys II, III, and IV*

Survey	National Wildlife Refuge	No. Geese Examined	Birds from which One or More Cultures were Not Contaminated		Birds from which One or More Noncontaminated Cultures were Positive for Trypanosomes	
			No.	%	*No.*	%
II	Pea Island, N. C.	59	58	98.3	12	20.7
III	Blackwater, Md.	48	44	91.7	9	20.4
IV	Blackwater, Md.	36	35	97.2	6	17.1
	Pea Island, N. C.	17	16	94.1	4	25.0
	Mattamuskeet, N. C.	14	11	78.6	0	—
	Turnbull, Wash.	11	11	100.0	0	—
	Back Bay, Va.	10	9	90.0	3	33.3
	Bombay Hook, Del.	6	6	100.0	0	—
	Sand Lake, S. Dak.	4	4	100.0	0	—
	Total Survey IV	98	92	93.9	13	14.1

trypanosome-infected birds were made on the basis of at least one culture being uncontaminated.

In Survey I cultures of bone marrow obtained at autopsy and grown in SNB-9 medium revealed presence of trypanosomes in 33 (40.2%) of 82 geese. Heart blood cultures grown in the same medium revealed flagellates in only 3 (3.9%) of 77 geese and 2 of these were also found positive in bone marrow cultures, the bone

marrow culture of the third being contaminated. No infections were revealed with the WB medium. Trypanosomes were not observed in any smears of bone marrow or heart blood from geese of this survey.

Survey II and Survey III, employing bone marrow cultures obtained from living birds and conducted within 9 days of each other, yielded similar results: trypanosomes were found in 12 (20.7%) of 58 geese in the one instance and in 9 (20.4%) of 44 geese in the other.

In Survey IV, the trypanosome incidence as demonstrated by bone marrow cultures taken from living birds varied from 0–33% for the several lots of geese examined, while of the total 92 geese in this series, 13 (14.1%) were positive. No trypanosomes were found in the heart blood of these birds.

DISCUSSION

There have been many surveys for trypanosomes in avian hosts based on microscopic examinations of stained films from either peripheral or heart blood. In an intensive study, Novy and MacNeal (1905) demonstrated the superiority of blood cultures over microscopic examination of blood films for the detection of these parasites. This has been confirmed by other workers (Bettencourt and Franca, 1906; Sergent and Sergent, 1907). Danilewsky (1889), the first to give an adequate description of avian trypanosomes, indicated that they were more common in bone marrow than in other tissues and subsequent workers have reported trypanosomes from bone marrow of birds when they were not demonstrable in blood (Petrie, 1905; Zupitza, 1909; and Minchin and Woodcock, 1911). Woodcock (1910) appears to have been the first to culture trypanosomes from avian bone marrow. He examined several species of birds in this manner, obtaining the marrow at autopsy.

Herman and Diamond (1952) reported negative results in the examination of 587 smears of heart and peripheral blood from 419 Canada geese obtained primarily from the Pea Island National Wildlife Refuge (ninety-one of these smears were from Survey I birds for which blood and bone marrow cultures were made). In addition smears from heart blood of the 98 birds constituting Survey IV also were negative for trypanosomes. In the examination of bone marrow smears made from each of the geese of Survey I, not a single intracellular or extracellular trypanosome was found although other species of blood parasites were observed.

Bone marrow culture appears to be the method of choice as a diagnostic procedure for the detection of trypanosomes in Canada geese. The advantages of bone marrow over heart blood were demonstrated by the results obtained in Survey I (Table I). In this survey bone marrow cultures showed that 33 (40.2%) of 82 geese harbored trypanosomes while heart blood cultures from 77 of these birds indicated that only three (3.9%) were positive. As previously noted 2 of the 3 birds found infected by blood culture also producted positive marrow cultures. The bone marrow culture of the third was contaminated.

Several possibilities may be suggested to explain the higher incidence of trypanosomes (40.2%) obtained in the first Pea Island survey (Survey I) as compared to that of the second Pea Island survey (Survey II) (20.7%). The difference could be attributed to the methods employed in obtaining the bone marrow in these surveys. In Survey I, the marrow was obtained at autopsy, in Survey II, from

living birds. Also, the divergence could have resulted from a seasonal variation reflecting activity of a blood sucking vector. Survey I was conducted in the latter part of April 1952, Survey II, in mid-February 1953. Still another explanation is the possibility that there was an actual reduction in parasite incidence between the 1951–52 and 1952–53 wintering seasons. In the latter connection it is interesting to note that the percentage of infections obtained in both Survey II (Pea Island National Wildlife Refuge) and Survey III (Blackwater National Wildlife Refuge) which were conducted within nine days of each other and in an identical manner were practically the same, 20.7% and 20.4% respectively.

SNB-9 medium was first devised by one of us (L.S.D.—unpublished data) for growing frog trypanosomes. Its successful use for growing *Trypanosoma cruzi* and *T. lewisi* led us to explore its possibilities as a suitable medium for the growth of avian trypanosomes. In addition to being excellent for culturing trypanosomes of Canada geese it has also been found to be useful for growing trypanosomes from the whistling swan (*Cygnus columbianus* Ord) and red-tailed hawk (*Bubo borealis borealis* Gmelin).

WB medium, employed for the culture of trypanosomes of frogs by Ponselle (1923) and by Diamond (1950) was adopted for use in Survey I after a single positive culture was obtained in it when used on the 5 geese of the preliminary test group (Herman and Diamond, 1952). The medium was dropped from the study when no further positive cultures were obtained in Survey I.

All developmental forms normally produced in trypanosome cultures were observed in our cultures from Canada geese. Leptomonad and crithidial forms predominated, leishmania and trypanosome types were rare. Since the vertebrate stage of the trypanosome necessary for proper species identification was not observed in tissues or fluids of our naturally infected geese, no specific determination was possible.

SUMMARY

1. Techniques are described for the cultural isolation of trypanosomes from avian bone marrow obtained from living birds or at autopsy. A new medium SNB-9 (saline-neopeptone-blood) is described. In addition to being a good medium for growing avian trypanosomes, it is excellent for growing trypanosomes of amphibians and mammals.

2. Evidence is presented demonstrating the superiority of (a) cultures over stained smears for detecting the presence of trypanosomes in the Canada goose, and (b) bone marrow over heart blood of this species as a source of trypanosomes for culture.

3. In April 1952, from cultures of bone marrow collected at autopsy it was demonstrated that trypanosome infection occurred in 33 (40.2%) of 82 Canada geese from the Pea Island National Wildlife Refuge. On February 17, 1953, cultures of bone marrow obtained from living birds revealed presence of trypanosomes in 12 (20.7%) of 58 geese from the same refuge. On February 26, 1953, by employing the latter method, 9 (20.4%) of 44 geese from Blackwater National Wildlife Refuge were shown to harbor the parasites. In another survey ninety-two geese from seven national wildlife refuges subjected to the biopsy technique showed evidence of infection in 13 (14.1%) birds and indicated that trypanosome infection is widely distributed in this host.

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STUDIES ON MARINE DIGENETIC TREMATODES OF PUERTO RICO. THE LIFE CYCLE IN THE FAMILY MEGAPERIDAE

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INTRODUCTION

The family MEGAPERIDAE Manter, 1934, is a small group of digenetic trematodes represented by only two genera and five species, all of which occur as adults in the intestine of marine fishes. The family was erected first by Manter (1933) as the EURYPERIDAE to contain the genera *Eurypera* and *Thysanopharynx* both of which were proposed at that time. Because the name *Eurypera* was preoccupied, Manter (1934) proposed the names *Megapera* and MEGAPERIDAE for the genus and family respectively. Later, (1947) he again gave that designation of the family

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as a new name although it was established as such in his 1934 note and must date from that time. It was not until his 1947 paper, however, that two of the four species he had assigned to the invalid genus *Eurypera* were formally placed in *Megapera.* The combinations *Megapera orbicularis* and *M. ovalis* accordingly would date from 1947 instead of 1934, as given in the later paper.

The group would seem to be a specialized one, limited to very few hosts and warmer seas. Linton (1907) described the first megaperid as *Distomum gyrinus* from two trunk fishes, *Lactophrys trigonus* and *L. tricornis* at Bermuda. Manter (1933) described the remaining four species in the family from fishes at Tortugas and reported *Megapera gyrina* (Linton) from that locality as well. Of the group, *M. ovalis* was the only species that he did not find in the trunk-fish, *L. tricornis,* the host of *M. ovalis* being the file or trigger fish, *Monacanthus hispidus.*

To date, there has been no account of a life cycle in the family MEGAPERIDAE other than an abstract of the present study (Cable, 1952). In a survey of marine cercariae at Tortugas, Miller (1925) described very briefly a species, *Cercaria K,* which seems certain to be the larva of a megaperid. It is very similar to the cercaria described below, develops in the same genus of snails, and was found in the locality where all the described species of the family are known to occur.

MATERIALS AND METHODS

All material used in this study was from Salinas Bay at Cabo Rojo. Mollusks were collected from shallow water with a scraper net and trunk fishes were obtained from traps nearby. The usual methods of studying larval and adult trematodes were employed with emphasis on living material. Measurements of cercariae were taken from heat-killed specimens handled in the manner described in detail in another paper (Cable, 1954). Figure 1 was drawn free-hand to scale from many such measurements, with details of the genital primordia added from whole mounts fixed in corrosive sublimate-acetic acid and stained with Semichon's carmine or Harris' hematoxylin. Other Figures, except Fig. 4, were drawn free-hand from living specimens.

OBSERVATIONS

Evidence concerning the life cycle

What is believed to be the life cycle of *Megapera gyrina* has not been demonstrated by means of controlled feeding experiments for which there was neither time nor adequate facilities. Evidence therefore is limited to information from the morphology of the cercaria and adult, and the ecology and habits of their hosts.

Among several species of cercariae found in dishes in which the common marine snail, *Cerithium variabile,* had been isolated was the unusual larva shown in Fig. 1. Further investigation revealed that this cercaria did not develop in *C. variabile,* but instead, in a minute species of *Crepidula* living attached to the shell of that snail. The cercaria could hardly be studied alive without its encysting and this occurred in an evidently normal manner in dishes of seawater after a period of swimming activity. This behavior suggested that in completing the life cycle, the cercaria encysts in the open and metacercariae are ingested by the definitive host, probably with vegetation. In this connection it may be observed that all such hosts

of the megaperids are herbivorous fishes. The possibility of a specific second intermediate host is not ruled out, however.

Perhaps the most suggestive evidence that the cercaria is a larva of the MEGAPERIDAE is afforded by its morphology which is strikingly like that of adult megaperids and very different from the structure of all other trematodes found in the locality from which, incidentally, a number of birds as well as many fishes were collected and examined for parasites. The several fundamental respects in which the cercaria and adult are alike are evident in the figures (cf. Figs. 1 and 4) and need not be enumerated at this point as they will be mentioned later in describing the cercaria.

Adults of the MEGAPERIDAE were obtained on several occasions from trunk fishes but until the collection is studied as a whole, the number of species belonging to that family will not be known. However, ecological evidence supports morphological data indicating that the cercaria described below is the larva of *Megapera gyrina* (Linton). That trematode was found in numbers only in *Lactophrys tricornis* trapped in Salinas Bay in the immediate vicinity of the molluscan host which has been tentatively identified as *Crepidula convexa*. Although very common in Salinas Bay, *C. convexa* was extremely rare elsewhere, although the other snails to which it is attached were among the commonest mollusks in many localities.

Description of Stages

Redia

Although several infected specimens of *Crepidula convexa* were opened and examined, only rediae producing cercariae were observed. However, cercariae had emerged from these snails and had younger infections been examined, earlier generations probably would have been found. The redia is simple and elongate but older ones are rather plump and may attain a length of almost 1.0 mm. when measured alive under no pressure. There is a short, sac-like intestine containing yellowish-brown pigment; the redia itself otherwise lacks noticeable pigmentation.

Cercaria (Figs. 1–3)

In structure and behavior, the cercaria resembles the trichocercous larvae of the LEPOCREADIIDAE. Instead of setae on the tail, however, there is a pair of lateral fins, which are notched and granular in structure, and a very low ventral fin along most of the length of the tail. Undulations of the lateral fins give them an appearance of being composed of setae joined by webbing as described by Miller (1925) for the similar species, *Cercaria K*, which he found to develop in a *Crepidula* at Tortugas. His species and the present larvae may be the same but the description of *Cercaria K* is too inadequate to decide the point.

The cercaria is biocellate and distomatous, with a very large oral sucker not at the extreme anterior end of the body, and a much smaller ventral sucker at about the mid-level. The prominent, spherical eye-spots are close to the oral sucker; other pigment is absent. The body is very thick, broadly rounded anteriorly with the maximum width at about the eye-spot level, and tapered toward the tail. The cuticle is thin and without evident spines or setae. Cephalic glands were not seen but the body is crowded from end to end with dense cystogenous glands with

granular and not rod-like cytoplasmic inclusions. The mouth is ventral and elongate. The prepharynx is short, very wide, and with longitudinal ribbing which evidently extends into, and lines the pharynx; it is evidently this structure of the prepharynx that accounts for the lobed anterior margin of the pharynx in the adult of not only *Megapera gyrina* (Fig. 4), but all other megaperids and at least one lepocreadiid as well (Manter, 1947, Fig. 28). The pharynx is massive and joins the ceca without the intervention of an esophagus. The ceca are greatly expanded, strongly arched anteriorly, and extend well toward the posterior end of the body.

Primordia of the reproductive system are well developed. There is an ovarian mass just posterior to the ventral sucker with a line of nuclei leading to the anterior margin of that sucker and a small testicular mass on each side just anterior to the arch of the cecum and on a level with the posterior end of the pharynx. The arrangement of the primordia thus is precisely the same as that of the gonads in the adult of *Megapera gyrina;* in figures of all other megaperids, the testes at least partially overlap the ceca.

The excretory system is complex and extremely difficult to observe because of the thickness of the body and the obscuring of details by the cystogenous glands. The flame cells and excretory tubules were best observed just after encystment of the larva and by manipulating it so that a lateral view was obtained. The excretory vesicle is Y-shaped as in adult megaperids; it has a long stem and short arms which are deflected anteroventrally. The vesicle contains highly refractile, laminated concretions; its wall is but slightly thickened and is uniformly granular without evident cellular structure, at least in living specimens. There is a well developed sphincter at the posterior end of the vesicle and an excretory chamber is visible at the junction of the body and tail; the position of the embryonic excretory pores was not determined. From the tip of each arm of the excretory vesicle, a main excretory tubule extends anteriorly almost to the oral sucker, then turns abruptly posteriorly and extends almost to the cecal arch where each tubule receives an anterior and a posterior collecting tubule. Each collecting tubule receives secondary tubules from three flame cell groups, making a total of six groups on each side of the body. The pattern was determined completely for only the second, third and fourth groups, all of which contained eight flame cells each but showed no precise uniformity in tubule and capillary pattern. It was determined that the anterior-most group also contained eight flame cells but two of their capillaries could not be traced with certainty. Although the fifth and sixth groups were even more difficult to observe, each was found to have at least six flame cells, making it probable that the excretory formula of the cercaria is $2[(8+8+8)+(8+8+8)]$.

Measurements in millimeters taken from heat-killed cercariae under no pressure are: body length 0.305–0.308, maximum width 0.113; tail 0.616–0.685 long and 0.038 in maximum width exclusive of lateral fins which attain a maximum width of about 0.040 and the ventral fin about 0.008. The oral sucker is 0.072–0.074 long and 0.077 wide, the ventral sucker 0.047 in diameter. The pharynx is 0.052 long and 0.040 wide; the diameter of the eye-spots is about 0.022.

The cercaria is a powerful swimmer, changing directions frequently in an erratic manner. It swims with the tail first, lashing in a figure-eight, but not as smoothly as in some cercariae. Larvae obtained by cracking the host were photo-

positive; it was not determined whether the species becomes photonegative after swimming for a time.

Spontaneously emerging cercariae were extremely difficult to study alive without their encysting on the slide and when heat-killed, much cystogenous material was exuded. After a swimming period the larvae encysted in the dish to form metacercariae which were spherical and measured 0.145–0.160 in diameter when alive and under no cover pressure.

Adult (Fig. 4 and 5)

Both Linton (1907) and Manter (1933) have described *Megapera gyrina* which is believed to be the adult of the cercaria reported here. Manter's figure is reproduced as Fig. 4 to bring illustrations of the various stages together in one plate. It will suffice here to supplement briefly their descriptions of the reproductive system. According to Manter, *M. gyrina* lacks a prostate, but living specimens under considerable pressure which deflected the seminal vesicle (Fig. 5), showed both a well developed pars prostatica and numerous, long prostatic gland cells. Parts of the female complex were so closely crowded and superimposed that details of the passages could not be determined in living specimens. A well developed Mehlis' gland was observed, however, and Laurer's canal was traced to its dorsal opening slightly to the left of the mid-line.

DISCUSSION

In discussing relationships, Manter (1933) concluded that the MEGAPERIDAE seemed to be more closely related to the lepocreadiids than to any other group. Later, (1947), he pointed out certain resemblances between the MEGAPERIDAE and CRYPTOGONIMIDAE, a family of the OPISTHORCHIOIDEA, but stated that the ani, body spines, pharynx, and excretory vesicle suggested relationship to the lepocreadiid genus *Bianium*. A close affinity between the MEGAPERIDAE and LEPOCREADIIDAE is strongly supported by the present study and the excretory system in the two groups is especially indicative of such a relationship. While a Y-shaped bladder is not common in the LEPOCREADIIDAE, it does occur there and the structure of the bladder wall is remarkably similar in the cercariae of the two groups. Although few lepocreadiid life cycles are known, it is evident that the tail of their cercariae is highly variable in respect to its ornamentation. Thus it is believed that differences in caudal structure with lateral setae in lepocreadiid larvae and fins in the MEGAPERIDAE are of minor importance. Offsetting these differences perhaps is the presence of a ventral fin in at least one cercaria in each group, the megaperid larva described here and the cercaria of *Deropristis inflata*, a lepocreadiid.

Differences between adults of the MEGAPERIDAE and LEPOCREADIIDAE concern chiefly the topography of the reproductive system. As a rule, the testes in lepocreadiids are more or less in tandem posterior to the ovary. Sometimes there are several testes and in *Microcreadium*, with two, they are side by side; although posterior to the ovary, their position in respect to each other is suggestive of the MEGAPERIDAE, as Manter observed. It may be noted that the megaperid genus *Thysanopharynx* seems to be intermediate between such a lepocreadiid genus as *Bianium* or *Microcreadium* and species of *Megapera*. In *Thysanopharynx*, the

testes are close together, barely anterior to the ventral sucker and well posterior to the pharynx, whereas in species of *Megapera* they are well separated and near the pharyngeal level.

Differences in topography of the reproductive system in the two groups seem to the writer to be perhaps no more than a subfamily distinction when one considers the effects that development of the extremely voluminous intestine in the MEGAPERIDAE might have exerted in displacing other structures. In fact, just such a displacement is strongly implied by the ventral deflection of the arms of the excretory vesicle, their being prevented from extending directly anteriad by the arches of the ceca and laterally by the thickness of the ceca which extend from near the dorsal to the ventral surface of the body. A displacement of structures is further indicated by the position of the ovary which is close to the ventral sucker in megaperids but some distance posterior to that sucker in lepocreadiids. A similar effect on the position of the testes could well result in the extreme situation seen in *Megapera gyrina* in which the testes are anterior to the arches of the ceca whereas in all other megaperids, the testes and ceca at least partially overlap.

SUMMARY

The cercaria of a megaperid, evidently *Megapera gyrina,* was found to develop in simple rediae in *Crepidula convexa* living attached to shells of other snails. The cercaria resembles the trichocercous larvae of the LEPOCREADIIDAE except that the tail has lateral fins instead of setae. After a period of swimming activity, the cercaria encysts in the open, indicating that a second intermediate host may not be essential to the completion of the cycle. Morphological evidence and ecological data bearing on the life history are presented and it is concluded that the MEGAPERIDAE are closely related to the LEPOCREADIIDAE.

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EXPLANATION OF PLATE

FIG. 1. Cercaria, entire in ventral view.

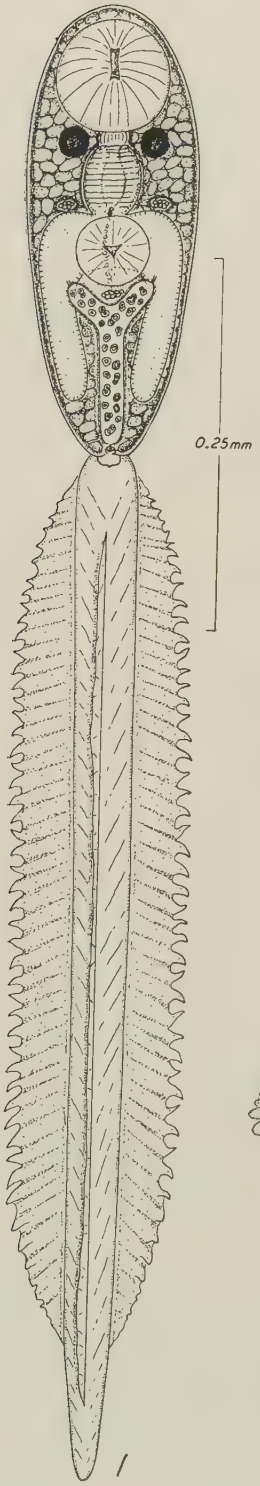
FIG. 2. Body of cercaria in lateral view showing excretory system of one side.

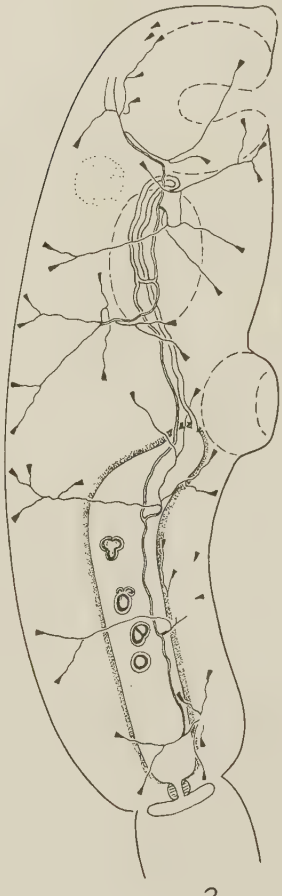
FIG. 3. Sketch of oral sucker, prepharynx and pharynx as observed in the living cercaria.

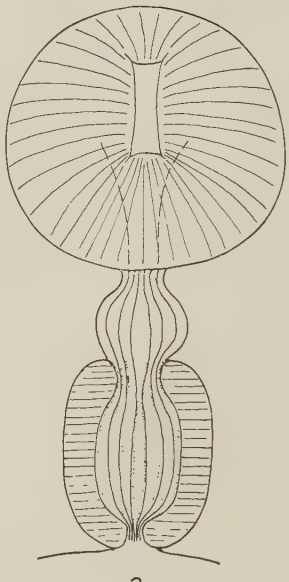
FIG. 4. *Megapera gyrina,* adult redrawn from Manter (1933).

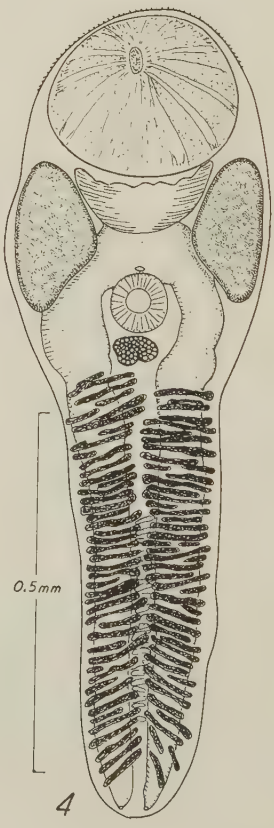
FIG. 5. *Megapera gyrina,* free-hand drawing of reproductive organs as observed in a living specimen under considerable pressure.

PLATE I









STUDIES ON *QUINQUESERIALIS HASSALLI* AND TAXONOMIC CONSIDERATIONS OF THE SPECIES OF *QUINQUESERIALIS* (TREMATODA: NOTOCOTYLIDAE)[1]

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During the summers of 1948 and 1951, more than 895 mammals collected by field parties of the University of Kansas, Museum of Natural History, were examined for helminth parasites. Collections were made in Kansas, Oklahoma, New Mexico, Colorado, Wyoming and South Dakota. 176 specimens of *Quinqueserialis hassalli* (McIntosh and McIntosh, 1934) Harwood, 1939 were collected as follows: 66 specimens from one *Microtus montanus caryi* Bailey, montane meadow mouse in Wyoming; 16 specimens from one *Microtus montanus fusus* Hall, montane meadow mouse in Colorado; 72, 2 and 16 specimens from three *Microtus pennsylvanicus modestus* (Baird), Pennsylvanian meadow mice, two in New Mexico and one in Colorado; 1 specimen from one *Thomomys talpoides ssp.*, northern pocket gopher in Wyoming.

The trematodes were killed and preserved in 5 per cent formalin. Toto mounts were prepared according to Riser's (1950) modification of Proescher's Celestin Blue B Stain. While none of the specimens were individually killed under a cover glass, they were all killed, preserved, and prepared in the same way. The NOTOCOTYLIDAE are not very muscular trematodes and although the ventral surface tends to become concave there is no great body contraction.

Genus *Quinqueserialis* Skvortsov, 1934

Quinqueserialis hassalli (McIntosh and McIntosh, 1934) Harwood, 1939. *Synonyms: Monostoma sp.* Stiles and Hassall, 1894; *Notocotylus hassalli* McIntosh and McIntosh, 1934; *Barkeria hassalli* (McIntosh and McIntosh, 1934) Szidat, 1936; *Quinqueserialis wolgaensis* Skvortsov, 1934.

Q. hassalli (Fig. 1) is a monostome trematode with more or less concave ventral surface on which occur five longitudinal rows of papillae. The intestinal ceca curve inward to pass medial to the testes. The two testes are lateral, near the posterior end of the body. An elongate cirrus sac opens at the median genital pore near the level of intestinal bifurcation. The ovary is median, between the testes. The vitelline follicles are in two lateral rows or groups, anterior to the testes. The uterus is pre-ovarian with lateral coils. An elongate metraterm is present.

Q. hassalli is a common trematode parasite found in the ceca of the microtine rodent *Microtus,* having been recorded only twice from other hosts: once from the jumping mouse (*Zapus*) by Erickson (1938) and once from the woodchuck (*Marmota*) by Rausch and Tiner (1948). The latter was reported with the notation that the animal was trapped in a wet marshy area and that remains of snails and vegetation were found in the stomach and intestine of the animal. It was suggested that

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[1] Studies from the Department of Zoology, University of Nebraska, No. 264. The work was done under the direction of Dr. H. W. Manter.

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this type food may have caused the infection. The reporting of the occurrence of *Q. hassalli* in the pocket gopher, *Thomomys talpoides ssp.,* constitutes a new host record.

Rausch and Tiner (1948) reported a 46 per cent incidence of this trematode in voles from the southern Wisconsin marsh. The trematodes averaged eight per infected host, with a maximum number of 37 in a single host. Kuns and Rausch (1950) reported a 14 per cent incidence of this trematode in "Willows and Wet Meadows" type habitat in the Jackson Hole Region of Wyoming, with a 4 per cent incidence for all types of habitats of that region. The prevalence indicated by my collections was much lower. Of 307 *Microtus* examined, a total of 5 was found infected or 1.6 per cent. Evidently local conditions of the habitat influences the distribution and incidence of infection. These conditions vary so greatly that figures of incidence are without much value. It may be noted, however, that the previously recorded maximum infection was 37 specimens. This is now extended to 72.

A study of the 176 specimens was made in order to determine morphological variations within the species.

Size and shape (Figs. 1 to 11): Specimens ranged from 1.6 to 3.96 mm. in length and from 0.88 to 2.04 mm. in maximum width. They were flat or with concave ventral surfaces. Some had longitudinal ridges on the dorsal surface (Fig. 11). Shape varied from elongate spatulate to pyriform.

It was possible to compare infections of varying intensities (72, 16, or 2 parasites) in the same species of host; and infections of equal intensity in different species of hosts. Infections of high intensity produced smaller specimens on the average. However, there were large specimens from heavy infections the same size as small specimens from light infections. No significant differences could be detected in specimens from the various hosts except that the single specimen from the pocket gopher (*Thomomys*) was largest in size and had minimal development of the ventral papillae.

Ventral papillae (Fig. 13): The structures found on the ventral surface have been called "glands" by some authors and "papillae" by others. Because the papillae do not have pores or characteristic glandular structures and because of the lack of any evidence that these structures are secretive, even in their beginning development, the term "papillae" seems preferable to "glands." The total number of countable ventral papillae per specimen varied from 74 to 96: lateral row, 15 to 20; paramesial row, 16 to 21; mesial row, 13 to 18. The papillary areas may become extended and invaded by internal organs such as the uterus, vitellaria, testes and ovary. In small bodied specimens, internal pressures due to competition of available space by developing organs may force these organs to invade the papillae and cause their further distortion or protrusion. In most cases, the papillae are simple knob-like structures. In some specimens they appear to be "two-headed" or shaped like a dumbbell. Rarely, the distal portion of the papillae are concave to give the appearance of a sucker. Some specimens have such small trace of ventral papillae that only by the most careful scrutiny could a few be revealed. In such cases it was difficult or impossible to determine the number of rows of papillae, nor could the number of papillae be ascertained. In most specimens, the five rows of papillae could be observed, but in these cases the number of papillae might or might not be countable. When countable, as mentioned previously, the number of papillae per

row may vary as much as five, e.g., from 13 to 18 or from 16 to 21. When not countable, the reason was there were areas within rows that had not developed papillae or papillae had fused together. This fusion of papillae was observed to occur both longitudinally and laterally and was due, in part, to the distorting effects caused by the invasion of the papillae by internal organs.

Internal morphology: The intestinal ceca of some specimens were simple tubes while in others they possessed very short lateral bulges. The ceca extend posteriorly approximately dorsal to the paramesial row of papillae, pass between the ovary and testes and end blindly a short distance from the posterior end of the body.

The two testes are extracecal, one on each side and posterior to the uterine coils. They measured from 0.24 to 0.72 mm. long by 0.18 to 0.54 mm. wide. The edges of the testes may appear wart-like or be fimbriated. The cirrus sac varies from 0.66 to 1.44 mm. long by 0.12 to 0.24 mm. wide. It is located in the anterior half of the specimen, and may be 1/2 or 1/3 the body length. The cirrus is heavily spined. The internal seminal vesicle is large and conspicuous in the posterior part of the cirrus sac. The ventral genital pore is located approximately at the level of the intestinal bifurcation. In most specimens it is slightly posterior to this point but in some it appears level with or anterior to the point of bifurcation.

The ovary is located between the testes, is somewhat longer than wide, usually irregularly lobed but sometimes almost smooth. It varies in size from 0.18 to 0.48 mm. long by 0.15 to 0.36 mm. wide. The anterior edge of the ovary may be anterior, on a level with, or posterior to the anterior level of the testes and similarly the posterior edge of the ovary may be anterior, on a level with or posterior to the posterior level of the testes. Mehlis' gland is preovarian with the posterior edges overlapping the anterior edge of the ovary. Vitelline follicles are extra-cecal and extend from near the anterior edge of the testes (or slightly posterior), anteriorly to a point varying from about 2/3 the distance to the cirrus sac, or to the base of the cirrus sac, or even slightly beyond. The vitellaria on both sides do not always extend the same distances. The vitellaria in some specimens appear as distinct globular follicles. They are not constantly in one, two, or more rows but vary greatly in distribution, size and shape. Individual follicles may fuse to form a mass of considerable size. The variations of the vitellaria are worthy of note since some authors use the size, shape and extent of the vitellaria as specific taxonomic characters in the genus *Quinqueserialis*.

The uterus consists of from ten to fifteen transverse loops extending laterally past the intestinal ceca and situated between the cirrus sac and the anterior end of the testes. There may possibly be more than a maximum of fifteen loops in some of the specimens; the uterus being so tightly packed and coiled that individual loops could not be determined. In some specimens uterine coils were between the vitellarian follicles and extended almost to the body wall. The development of the uterus and its extension laterally past the intestinal ceca is a constant and prominent characteristic of *Q. hassalli*. An elongate, glandular metraterm is present which varies in length from 0.42 to 1.02 mm. The metraterm may be 1/2 or as long as the cirrus sac.

The eggs (Fig. 12) are ovoid and vary in size from 0.012 to 0.022 mm. long by 0.008 to 0.016 mm. wide. The two polar filaments found on all ova emerging from the uterus of mature specimens were also observed on ova within the anterior

loops of the uterus. In some specimens, young ova within the posterior uterine loops were observed without filaments or with short rudimentary filaments.

DISCUSSION

Four species of *Quinqueserialis* have previously been considered valid: *Q. quinqueserialis* (Barker and Laughlin, 1911) Harwood, 1939; *Q. hassalli* (McIntosh & McIntosh, 1934) Harwood, 1939; *Q. wolgaensis* Skvortsov, 1934; and *Q. floridensis* Rausch, 1952. *Q. quinqueserialis* is commonly found in large numbers in both muskrats (*Ondatra*) and voles (*Microtus*). *Q. hassalli* has been reported from only voles (*Microtus*) with the two exceptions previously noted. *Q. wolgaensis* was described from *Arvicola* (= *Microtus*) in Russia. *Q. floridensis* was described from the round-tailed muskrat (*Neofiber*) in Florida.

The differentiation of these species has previously been based on number of papillae in ventral rows; form and distribution of vitellaria; length of metraterm in relation to length of cirrus sac; and egg size. This study has shown that all of these characters show considerable variation.

Other workers on species of notocotylids have disagreed on the importance of ventral papillae as a differentiating character. Braun (1892) and Lal (1935, 1936) stressed the importance of the number of rows of papillae, the number of papillae per row and the protrusibility of the papillae. However, Looss (1899), Baylis (1928), Duthoit (1931), Noble (1933), and Harwood (1939) disagree as to the importance of such characters. My conclusions were that since irregularities are found in the number of ventral papillae, this character is unreliable and should be used with the greatest discretion only when a large number of specimens are available for study.

Harwood (1939) and Ruiz (1946) in their reviews of the genus *Quinqueserialis* use characters which by this study are not satisfactory in differentiating species. Harwood (1939) and Rausch (1952b) have suggested that *Q. quinqueserialis, Q. wolgaensis* and *Q. hassalli* might actually be a single species.

Q. quinqueserialis is similar to *Q. hassalli* in many respects. However, in *Q. quinqueserialis* the transverse uterine coils do not extend laterally past the intestinal ceca and in *Q. hassalli* the uterine coils do extend laterally past the intestinal ceca and may extend into the area of the vitellaria to the body wall. This character which has not been stressed previously by authors appears to be a clear and constant difference between these two species. It is suggested that this character be considered the basis on which to distinguish the two species instead of vitellaria which have been shown to be too variable.

Q. wolgaensis was differentiated by Skvortsov (1934) by the shape of the body, the number of papillae, and the size of the vagina. Skvortsov was apparently not aware of *Q. hassalli* when he described *Q. wolgaensis.* He stated that only one American species, *Notocotylus quinqueserialis,* had five rows of ventral papillae. Morphological characters of *Q. wolgaensis* are identical to ones found in my specimens of *Q. hassalli.* The uterine coils of *Q. wolgaensis* as figured by Skvortsov, extend laterally past the intestinal ceca. Thus, *Q. wolgaensis* is considered a synonym of *Q. hassalli.*

Q. floridensis was described by Rausch (1952a) from the round-tailed muskrat, *Neofiber alleni nigrescens* Howell. He considered the relatively extreme lateral

extent of the uterine coils beyond the intestinal ceca, the absence of polar filaments of the eggs, restricted longitudinal extent of the vitellaria, and small body size to be unique and sufficient to distinguish his species from other species of *Quinqueserialis.*

A similar extreme lateral extent of the uterus does occur in *Q. hassalli* but not *Q. quinqueserialis.*

Through the courtesy of Dr. Robert Rausch, I have had the opportunity to examine specimens of *Q. floridensis.* In these specimens, I was able to observe the polar filaments of the eggs. Some of the eggs and their polar filaments are identical in shape and size to those of *Q. hassalli.* The length of the body and the size of the sucker of *Q. floridensis* are approximately the same as the smaller specimens of *Q. hassalli.* The testes, cirrus sac, ovary, metraterm are (proportionally) approximately one-half of the sizes of those found in the smaller specimens of *Q. hassalli.* The chief distinguishing character of *Q. floridensis* appears to be the extent of the vitellaria which are located almost wholly posterior to the uterine loops. *Q. hassalli* has vitellaria located in a longitudinal row, posterior and also lateral to the uterine loops. The shortest extent of vitellaria in *Q. hassalli* is similar to, but not identical with that of *Q. floridensis.*

The status of *Q. floridensis* as a valid species, based only on small body size and extent of vitellaria, seems to the author to be somewhat uncertain. Rausch (1952b) stated that in *Q. quinqueserialis* the specimens from the muskrats are relatively smaller than ones from voles. Since *Q. hassalli* has not been described from any muskrat, it may be that *Q. floridensis* are such specimens and that the differences are a result of host effect. However, *Q. floridensis* must remain a distinct species until feeding experiments or life cycle studies can show otherwise.

Differentiation of the three species of *Quinqueserialis* can be made as follows:

KEY TO THE SPECIES OF *Quinqueserialis*

1. Transverse loops of uterus not extending laterally past intestinal ceca .... *Q. quinqueserialis*
   Transverse loops of uterus extending laterally past intestinal ceca 2
2. Vitellaria extend from testes anteriorly to a point 2/3 or more the distance to the cirrus sac. *Q. hassalli*
   Vitellaria almost wholly posterior to uterine loops ..... *Q. floridensis*

Another species of NOTOCOTYLINAE resembles *Q. hassalli* except for ventral papillae. *Notocotylus noyeri* Joyeux, 1922, from *Arvicola* (= *Microtus*) was described as possessing only three rows of papillae. The similarity of this species to *Q. hassalli* plus the uniqueness in its taxonomic position are such that the possibility of its specific identity to *Q. hassalli* should be considered.

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EXPLANATION OF PLATE

All figures are of *Quinqueserialis hassalli.* They were all (except 11 and 13) drawn with the aid of a camera lucida, minor details and corrections being supplied freehandedly. Figures 11 and 13 are diagrammatic. Abbreviations: *ce,* cecum; *cs,* cirrus sac; *dr,* dorsal ridge; *met,* metraterm, *ov,* ovary; *t,* testes; *ut,* uterus; *vp,* ventral papillae; *vt,* vitellarium.

FIG. 1. Specimen from the pocket gopher, *Thomomys.*

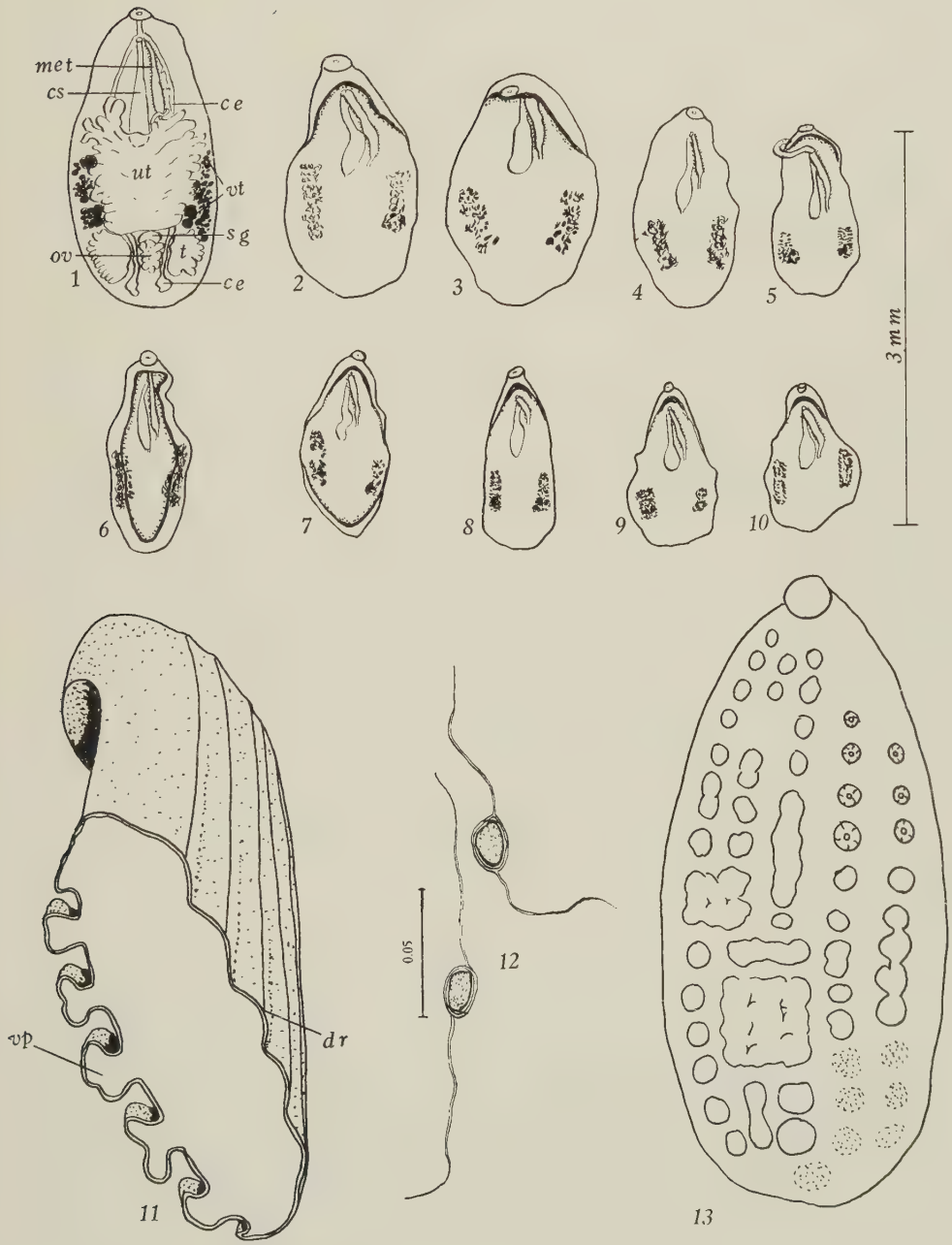
FIGS. 2 to 10. Specimens from species of meadow mice, *Microtus,* to show variation in size and vitellaria.

FIG. 11. A stereogram, showing the body form of the anterior half of a specimen with ventral papillae and dorsal ridges.

FIG. 12. Mature eggs with filaments.

FIG. 13. Diagram, showing all possible anomalous forms of ventral papillae: absence; rudimentary; sucker-like; double-headed; and fusion of two or more papillae.

PLATE I



PREVALENCE OF *DIROFILARIA IMMITIS* IN MEMPHIS, TENNESSEE[1]

Don E. Eyles, Colvin L. Gibson, Frances E. Jones and M. E. G. Cuningham

Memphis, Tennessee, lies within the known range of *Dirofilaria immitis;* however, there have been no reports from the area of the frequency of this parasite and the disease it causes. Otto (1949) has suggested that disease-producing levels of *D. immitis* infection are found only along the Atlantic seaboard and the Gulf coast, but he also pointed out that in the South the distribution of the parasite has not been well defined.

The present investigation was designed to determine how frequent *D. immitis* infection is in pound dogs of Memphis and vicinity.

METHODS

The dogs utilized were strays picked up in the City of Memphis and in Shelby County, and were obtained from the Memphis dog pound. Most of the dogs came from the City, but the origin of individual dogs could not be ascertained.

During the first part of the study the dogs were examined for microfilariae only by direct microscopic examination of a drop of blood. In later work the dogs were examined for microfilariae and for adult heart worms. In this case a little over one cc. of blood was taken from the femoral vein of the dogs prior to sacrifice using a small amount of heparin to prevent coagulation. For direct examination 10 cmm. of blood was placed on a microscope slide and the number of microfilariae counted using the low power objective. If no microfilariae were found, the remaining one cc. of blood was laked with saponin. Following this, the blood was centrifuged and the sediment examined for microfilariae.

To find the adult worms, the hearts and lungs of the dogs were removed in a block after the dogs were sacrificed. Following this, the right ventricle was opened as was the pulmonary artery. Dissection of the pulmonary artery was carried down to the small branches in each lung lobe. Any adult worms found were preserved for study.

RESULTS

Prevalence of D. immitis: From May, 1944 through December, 1945 local pound dogs were sampled in order to find infected dogs for chemotherapeutic studies. During the 20 month period the blood of 618 dogs was examined directly for microfilariae, an average of 34 per month (on a basis of 18 months as no dogs were examined in October, 1944 and January, 1945). Of the 618 dogs, 94 were found infected (15.2 per cent). No adult worm data were obtained.

From September 9, 1952 through January 29, 1953, 204 additional dogs were

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[1] Contribution from the U. S. Dept. of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases located at the Medical School of the University of Tennessee, 874 Union Avenue, Memphis 3, Tennessee. Acknowledgement is due Dr. R. A. Woodbury and staff associates of the Medical School of the University of Tennessee for active cooperation in this investigation.

examined by direct blood examination, concentration, and dissection of the heart and pulmonary artery. Forty-four (21.6 per cent) were found to be infected with some stage of *D. immitis*. Microfilariae were found in blood samples taken prior to death in 42 of the dogs (20.6 per cent). In 23 instances the microfilariae were seen directly and in 19 instances only after concentration. Ten of the dogs (4.9 per cent) showed adult worms in the right ventricle or in the pulmonary artery at autopsy; two of these were negative for microfilariae.

From the above two samples it is apparent that dogs of Memphis and vicinity are frequently infected with the microfilariae of *D. immitis,* but much less frequently carry adult worms in the heart. This is most likely due to the long persistence of microfilariae after the death of adult worms, which has been reported by some investigators. To some extent, the large number of dogs with only microfilariae may be a reflection of intermittent and low potential transmission. It is obvious that the dogs which showed only microfilariae at one time had been parasitized by adult worms of both sexes. If we do not consider the possibility of localization of worms in some site other than the heart, we must consider that spontaneous disappearance of adult worms has taken place in all of these dogs.

Table 1 compares the findings of our second sample with those of Brown (1939) and Mundhenk and Greene (1939), who also reported prevalence on the basis of both microfilariae and adult worms; and with the study of Ward and Franklin, who reported adult worms only. Although possible seasonable variation makes cautious comparison necessary, the data of Brown and of Ward and Franklin (1953) obviously reflect situations in which the transmission potential was high compared with that in Memphis. In Memphis the majority of the dogs examined had microfilariae only;[2] whereas, most of the dogs in Savannah and presumably Clarksdale, Mississippi had adult worms also. This may be attributed in part to a longer season of transmission in the Georgia city, since the climate there is much milder than in Memphis. In addition, mosquito density in general is much higher in the Savannah region and the Clarksdale, Mississippi region due to the greater extent of mosquito breeding waters.

Otto (1949) considered that the high prevalence of *D. immitis* in Savannah and other coastal cities indicates that a salt marsh mosquito (possibly *Aedes sollicitans* or *A. taeniorhynchus*) is involved in transmission. This may appear logical; however, it is also true that many fresh water mosquitoes are more abundant near the coast due to the great extent of surface water in such regions. This is particularly true in the vicinity of Savannah where drainage is poor and a high water table exists.

Further research on the mosquito vectors is needed before drawing any conclusions as to the principal transmitters of the infection; however, salt marsh mosquitoes could not be responsible for the moderately high prevalence of *D. immitis* in Memphis. Likewise, salt marsh mosquitoes could not be held responsible for the very high prevalence of *D. immitis* in Clarksdale, Mississippi. The dogs of this city, which is several hundred miles inland, actually showed a higher prevalence of adult worms than the dogs of coastal Savannah, Georgia.

[2] The paper cited by Ward and Franklin (1953) does not mention the locality in Mississippi from which their dogs originated. Through personal communication we learned that the locality was Clarksdale.

Among other possible vectors, *Anopheles quadrimaculatus* should be considered. It is very abundant in the Mississippi delta region where Clarksdale is located. It also occurs frequently in the Memphis and Savannah regions. Phillips (1939) and Kartman (1953) showed that this species is an efficient experimental host of the parasite. On this basis, its importance as a vector in the field should be investigated.

The findings of Mundhenk and Greene (1939) are difficult to interpret. From their data it would appear that *D. immitis* is very frequent in the vicinity of Auburn, Alabama as evidenced by the finding of microfilariae, but that adult worms are extremely rare. Whether or not the possibility of postmortem migration of adult worms was considered was not mentioned. Recent experience at Auburn, Alabama (personal communication from Dr. W. S. Bailey) has been that about 37 per cent of the clinic dogs at the School of Veterinary Medicine have microfilariae in the peripheral blood (using concentration methods for examination), a figure more in accord with those of other observers than that of Mundhenk and Greene No adult worm prevalence data are available for comparison.

Number of microfilariae found in the blood and correlation with adult worm burden: The median microfilarial count of the 23 dogs found positive by direct

TABLE 1.—*Comparison of the results of the present study with other studies. Mf. = microfilariae*

	Locality in which study was done			
	Memphis, Tenn. (present study)	Savannah, Ga. (Brown, 1939)	Auburn, Ala. (Mundhenk and Greene, 1939)	Clarksdale, Miss. (Ward and Franklin)
Number of dogs examined	204	186	200	153
Per cent with mf. only (A)	16.7	9.6	..	..
Per cent with adults only (B)	1.0	4.8	..	..
Per cent with both mf. and adults (C)	3.9	19.3	..	..
Per cent with microfilariae (A + C)	20.6	29.9	76	..
Per cent with adult worms (B + C)	4.9	24.6	1	32.7
Total per cent infected (A + B + C)	21.6	33.7	76	..

examination was 600 per cc. The arithmetic mean number was 2,300. This is evidence of marked positive skewness, and the use of the logarithmic mean would therefore best express the central tendency. The log mean was found to be 2.8 corresponding to a count of about 600 microfilariae per cc.—the same as the median.

In making comparisons between samples, statistical analysis based on the log of the microfilarial count should give the most reliable results as the logarithmic transformation renders the distribution more nearly normal. In a later section of this paper we use this method to compare samples from the Memphis area, taken at different times of the year. Similar comparisons cannot be made with previously published work because the essential statistics are lacking.

It was obvious from our data that the dogs with the highest microfilarial counts tended to be the dogs that had adult worms in the heart; for example, the four dogs with the most microfilariae also had adult worms. A Pearsonian correlation coefficient was calculated using the log of the microfilarial density plus one and the log of the adult worm count plus one. This gave a result of $r = +0.447$ which is a moderately high degree of positive correlation between microfilarial density and adult worm density.

Mean adult worm burden based only on the dogs showing adults was 7.2. Female worms outnumber males by about 3 to 2.

Seasonal variation in prevalence of D. immitis: The prevalence data from the initial 20-month sampling by direct blood examination only, showed an interesting seasonal variation.

Figure 1 presents the month to month proportions infected. It is evident that the prevalence of microfilariae in the peripheral blood, as measured by this sampling, was much greater during the warmer months than during the cool months. From November to February scarcely any infected dogs were found, whereas the proportion infected during the summer months ran as high as 20 to 30 per cent. This is in substantial agreement with the findings of Kubo (1938) who noted a very pronounced seasonal periodicity in China with a maximum of microfilariae in the peripheral blood towards the end of August and a minimum between November and December.

Several hypotheses can be advanced to explain this apparent seasonal variation. In the first place we may be dealing with a real periodicity. In Memphis, trans-

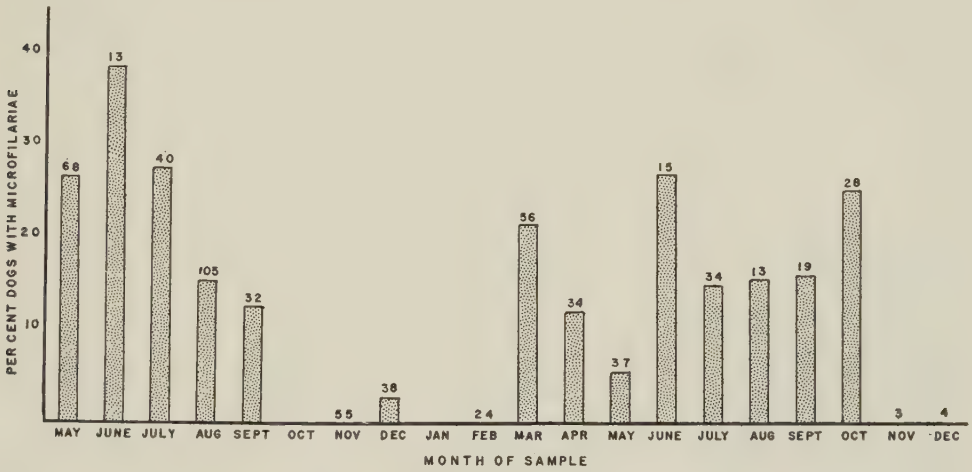

FIG. 1. Prevalence of *Dirofilaria immitis* as shown by monthly direct blood examinations for microfilariae in the peripheral blood of Memphis dogs. The numerals over the columns refer to the number of dogs examined.

mission by mosquitoes could not be continuous due to the severe winter period, and it may be that infection occurs periodically and that the life of adult worms is shorter than is generally believed, resulting in dogs with light or no infections in the winter.

Secondly, weather conditions may cause the microfilariae to leave the peripheral circulation. Last but unlikely, it is possible that the winter and summer dogs came from different areas served by the pound. Most of the dogs were bled in the morning in this study so changes in the time of day of sampling could not account for the seasonal variation.

In order to study this possible seasonal variation more thoroughly, the series of 204 dogs examined by autopsy was divided into two groups of 102 each. The first series was autopsied between September 10 and October 9, 1952 and will be referred to as the fall sample. The other 102 dogs were autopsied during the period between December 2, 1952 and January 31, 1953, and this will be called the winter sample.

Table 2 compares the data obtained from the two groups. Actually, more infected dogs were found in this winter sample, but the difference was not statistically significant; however, fewer dogs in the winter sample were positive by direct examination for microfilariae (our 20-month sample was examined by the direct method only). The density (mean log) of microfilariae in the winter sample was less, the difference being statistically significant ($p = 0.04$). Likewise the mean of the log of the adult worm number per infected dog was lower, and again statistical significance could be demonstrated ($p = 0.0007$). An interesting difference between the two samples was that a high degree of positive correlation ($r = +0.811$) between adult worm number (log plus one) and microfilarial density (log of count per cc. plus one) was demonstrated for the fall sample as compared with no correlation ($r = -0.130$ which is not significantly different from zero) for the winter sample. Statistical study shows that the difference is significant ($p = 0.04$ using Z-transformation).

In summary, the more thorough study by direct blood examination, concentration, and autopsy demonstrated statistically significant differences between the fall and the winter samples. Although the extreme seasonal variation evident in the

TABLE 2.—*A comparison of the prevalence of* Dirofilaria immitis *in fall and winter samples from Memphis, Tennessee. Mf. = microfilariae*

	Fall sample	Winter sample	P-value
Number of dogs examined	102	102	
Total number infected—adults and mf.	19 (18.6%)	25 (24.5%)	P = Circa 0.32
Total with mf.	19	23	
Mf. found on direct examination	13	10	
Mf. found on concentration only	6	13	
Adult worms present	5 (4.9%)	5 (4.9%)	
Adult worms only present	0	2	
Log mf. count, mean	2.9 ± 0.23	2.1 ± 0.32	P = 0.04
Log adult worm count, mean	0.99 ± 0.116	0.20 ± 0.20	P = 0.0007
Correlation (r) of adult worm burden and density of mf.	+ 0.811	− 0.130	P = 0.04

initial sample studied by direct blood examination only was not observed, lower microfilarial counts and lower adult densities were noted in the winter. Technical differences, year to year meteorological variations, or other factors may be responsible for the partially discrepant findings. The desirability of obtaining further sample in other seasons is indicated.

SUMMARY

Direct blood examination of 618 dogs from the Memphis-Shelby County, Tennessee dog pound over a 20-month period showed an average prevalence of 15.2 per cent infection with microfilariae of *Dirofilaria immitis*. An additional sample of 204 dogs was examined by direct blood examination, concentration of blood, and by dissection of the heart and pulmonary artery. Microfilariae were found in 20.6 per cent of the dogs, and 4.9 per cent showed adult worms in the right ventricle of the heart and the pulmonary artery.

Median microfilarial density (not counting dogs found positive only after concentration of blood) was 600 per cc. Mean adult worm burden was 7.2 per infected dog. There was a moderately high positive correlation between microfilarial density and adult worm burden.

The data obtained from the 20-month sample showed a marked seasonal variation. Examination of the more complete data from the sample of 204 dogs disclosed that dogs autopsied in the winter had lower microfilarial and lower adult worm densities than fall dogs, but about the same proportions were found infected. The partial discrepancy presented by these findings has not been resolved.

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REPORT ON A HUMAN CASE OF *ENDAMOEBA POLEČKI* PROWAZEK, 1912

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INTRODUCTION

Upon examining the feces of an Egyptian assistant at the U. S. Naval Medical Research Unit No. 3 in Cairo, numerous *Endamoeba* trophozoites and a few cysts were detected in a routine normal saline preparation. Presumptively diagnosed as *Endamoeba histolytica,* hematoxylin-stained preparations revealed *Endamoeba* trophozoites combining some of the characters of both *E. histolytica* and *E. coli.* The cysts were uninucleate, contained an abundance of chromatoid material, and in many a dark staining inclusion mass appeared in the cytoplasm. Since the consistency of the stool specimen was mushy to liquid, it did not seem strange that uninucleate cysts predominated in the presence of large numbers of trophozoites. In view of certain morphological peculiarities, another specimen was examined a few weeks later. The stool appeared formed, yet the saline direct smear examination revealed numerous uninucleate cysts the size of *E. histolytica* (large race), but with an abundance of refractive chromatoid substance and other atypical features. Hematoxylin preparations again revealed morphological characteristics atypical of either *E. histolytica* or *E. coli.*

A review of the literature indicated that the characteristics of this parasite corresponded with those of the amoeba described by Kessel and Johnstone (1949) un-

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der the name *Endamoeba polecki* Prowazek, 1912, from man and imported Indian monkeys in the United States, and which was later reported by Lubinsky (1952) from a man in Pakistan. Although there is some uncertainty about the correct name for this parasite, it will be referred to in the present paper as *Endamoeba polecki* for reasons given in the discussion at the end of this paper. The morphological characteristics of the parasite observed in this study will be discussed in an effort to provide additional information on the taxonomic status of this parasite and to compare it with the previously reported human cases of the same or closely related species.

MATERIAL AND METHODS

This case of *Endamoeba polecki* in a 26 year old Egyptian male, was under observation from November 1949 to August 1952, during which time the subject was available for study. He was born in the Sinai district of Egypt, and during his first seven years was associated with domestic animals such as camels and goats. He later lived in Palestine for 15 years, at which time he was associated with horses and donkeys. Since 1948, the subject had not been in close proximity with domestic animals that might account for the transmission of the *Endamoeba polecki* infection. Of 44 individual stool samples examined for the presence of this species, only seven failed to reveal this amoeba. Saline direct smear examinations were made on all specimens, and permanent preparations were made from 32 of the 44 samples submitted. Staining was with Heidenhain's iron hematoxylin, after fixation with Schaudinn's fluid (5% glacial acetic acid added). Lugol's iodine and "MIF" direct smear staining (Sapero, Lawless and Strome 1951) was done on stool samples that revealed large numbers of cyst forms. Attempts were made to obtain cultures using Bacto Endamoeba medium (Difco). A protoscopic examination of the subject was made. Two purged specimens were obtained at the completion of the study. Various drugs recommended for the treatment of *E. histolytica* were administered to the subject before and during the two and a half year study.

RESULTS

In the course of the observation, the following species of intestinal Protozoa in addition to *E. polecki* were encountered: *Endamoeba coli,* a small race of *E. histolytica, Endolimax nana, Iodamoeba butschlii, Dientamoeba fragilis, Giardia lamblia, Chilomastix mesnili,* and *Enteromonas hominis.* Ova of three species of helminths, *Hymenolepis nana, Enterobius vermicularis,* and *Trichuris trichiura,* were recorded.

A reasonable explanation can be offered for the noticeable absence of the large race *E. histolytica* in this study. Just prior to the detection of *E. polecki,* the subject received six injections of emetine, a year later a course of Diodoquin was administered, and a few months prior to the completion of the study, a course of Vioform was taken. These treatments had no apparent effect on the course of the *Endamoeba polecki* infection, and there was no change in the general morphological characteristics of the parasite. At the termination of the project, purged specimens produced the heaviest density of *E. polecki* cysts and trophozoites noted during the study. Kessel and Johnstone (1949) reported that in their studies they observed that *E. polecki* in monkeys does not respond to amebacidal treatment as does *E. histolytica.*

A proctoscopic examination made during a bacillary dysentery episode revealed no significant finding. No ulcerated areas were observed, and other than the irritation caused by the bacterial infection the intestinal mucosa appeared normal. Sulfa drugs were administered and the patient recovered. During the period of observation this was the only gastro-intestinal complaint experienced by the subject. With the exception of this episode, the character of the stool remained constant throughout the study ranging from formed soft to formed firm in consistency with no evidence of blood or excessive mucus. No discomfort could be attributed directly to the presence of the *E. polecki* infection.

Living trophozoites and cysts were observed in normal saline preparations. Trophozoites measured from 10 to 24 microns when rounded, with an average of about 16 microns. The motility in the living trophozoites resembles that of *Endamoeba coli,* as was pointed out by Kessel and Johnstone (1949). Occasionally a few trophozoites assumed a momentary directional flow characteristic of *E. histolytica,* but such activity did not persist. The cytoplasm appeared coarsely vacuolated, and contained an abundance of ingested bacteria, yeast, and lower fungi, but did not contain other protozoa or ingested red blood cells. The refractile outline of the nucleus was occasionally visible. Even in the presence of a long, extended pseudopod, the endoplasm usually retained a spherical shape, as described by Lubinsky (1952). Occasionally pseudopodia were projected from one or more areas in an explosive manner, closely followed by an immediate rounding up of the parasite. In the freshly passed stool, living trophozoites appeared to disintegrate rather quickly at room temperature.

The living cysts of *Endamoeba polecki* appeared highly refractile, round or slightly oval, and usually contained an abundance of chromatoid bodies lying in pairs parallel to the cyst wall and in the outer cytoplasmic layer. The living cysts measured between 10 and 16 microns with an average of about 12 to 13 microns. In some examinations, however, only sparse chromatoid substance was noted. Formed firm to hard stools produced cysts containing less abundant chromatoid substance. The inclusion mass within the cysts, as reported in the human cases by Kessel and Johnstone and Lubinsky, failed to take the iodine reaction common to protozoan species containing glycogen. This also agrees with observations of this species from pigs, monkeys, and other animals, first mentioned by Cauchemez (1922).

Specimens containing large numbers of *E. polecki* cysts were placed in a culture medium (Bacto-Endamoeba medium, Difco) which is an excellent medium for the cultivation of *E. histolytica.* Only a few subcultures were obtained. With the addition of penicillin as suggested by Kessel and Johnstone, 10 subcultures were maintained, but the colony gradually disappeared. Excystment of cyst forms was noted as early as four hours after culture inoculation.

Living trophozoites from culture appeared much the same as in the fresh stool. Nearly all specimens ingested great quantities of rice particles. The motility, however, appeared somewhat changed in that several trophozoites were quite active. Some even assumed the progressive action of *E. histolytica,* although a hyaline blade appearance of the pseudopodia was observed in only a few. The cytoplasm appeared less coarsely vacuolated, and the nuclear ring was usually not visible. The motility of the culture trophozoites compared favorably with the findings of Kessel

and Johnstone but, as they state, it is of little practical diagnostic value to compare trophozoites of amebae occurring in stools with those growing in culture.

CHARACTERISTICS OF *E. polecki* IN STAINED IRON HEMATOXYLIN PERMANENT MOUNTS

Six hundred and seventy-five cysts were carefully studied in 27 stained stool specimens. The size ranged from 8.3 microns to 14.94 microns with an average of about 11.62 microns. The shape was nearly always round, but was occasionally elongated. The cytoplasm was finely granulated, foamy, and light in color. Glycogen was or was not present, occasionally even in the presence of the inclusion mass. Eighty-four of the 675 cysts examined contained distinct glycogen areas and 346 contained an inclusion mass. This mass appeared as a peculiar, dark staining area of cytoplasm condensed in the middle of the cyst. Although more or less spherical in shape, the outlines were usually not sharply defined, and were similar to the "mass chromophile" depicted by Brumpt (1949) in typical cysts of *E. histolytica*. Kessel and Johnstone speculated that this inclusion mass may be a stored food substance used by the cyst as it ages. Chromatoid substance was usually abundant, the bodies rod shaped or spherical with angular or rounded ends, occasionally pointed and rarely thread-like in appearance. They were located in the outer cytoplasmic layer, parallel to the cyst wall, and were usually not less than one or more than four microns in length. A total of 539 of the 675 cysts examined contained chromatoid substance. The nuclear diameter was about one-quarter to one-third that of the cyst. The karyosome was large and spherical or stellate in shape, solid, or consisting of aggregated granules; or it was in the form of a small dot or clustered group of small dots. The larger massed type karyosome occupied half to three-quarters of the nuclear area and was usually central, rarely eccentric. The small dot-like karyosome or those consisting of a group of small dots, were central or eccentric in position. A few granules may surround the small dot-like karyosome, giving the appearance of a halo. The type of karyosome containing one or a cluster of small dots was encountered frequently in those cysts that had lost the inclusion mass, whereas in cysts containing the latter structure, a large massive, or stellate shaped karyosome was usually seen. The karyosome may be connected with the nuclear membrane by radial chromatic fibers, although often these are not observed. A layer of fine chromatin granules is encrusted on the nuclear membrane, causing it to appear as a dark ring in optical section. The chromatin material may be evenly encrusted or arranged in beads or plaques. These granules often form a thicker layer at the poles of the nucleus, lending the appearance of a crescent as mentioned by Lubinsky. Of the 675 cysts closely examined, seven were binucleated, and of the thousands observed over the period of observation, one appeared quadrinucleate.

One hundred trophozoites were carefully studied. These measured, when rounded, 11.62 to 22.51 microns, the majority falling between 14.94 and 16.6 microns. The cytoplasm was coarsely vacuolated, well defined, and contained ingested particles of various nature. It was fine but dense in texture, producing a relatively granular appearance. In many, a distinct line of demarkation existed between endoplasm and ectoplasm. Often the periphery of the ectoplasm was irregular in outline. The nuclear diameter was 2.5 to 5.0 microns. The karyosome was small,

occasionally dispersed into two or three small granules; it may be central or slightly eccentric in position. The peripheral chromatin formed a layer of minute granules, giving a fine beadlike appearance with usually a few larger granules interrupting the regularity of the contour of the nuclear ring. These granules may be more densely accumulated at one or both poles of the nuclear ring. Kessel and Johnstone's report on *E. polecki* in monkey and man illustrates this in the figure of trophozoites from a monkey. The space between the karyosome and nuclear membrane in this type of nucleus, in contrast to the type with a large massive karyosome often seen in cysts, usually stains more deeply than the endoplasm in both cyst and trophozoite stages.

EXAMINATION OF CERTAIN OTHER PERSONS AND ANIMALS

One hundred individuals working in pig-raising communities in Egypt were selected for study and examined for the presence of this parasite. The findings were negative. Amebae which morphologically resembled the *Endamoeba polecki* studied in this human case were obtained from several pigs.

DISCUSSION

Endamoeba with uninucleate cysts have been observed in a great variety of animals, including man, monkeys, pigs, cattle, sheep, goats and a number of wild species of ungulates. These amebae also agree with one another in other characteristics, which may show a considerable degree of variation, but the range is remarkably constant when any large series is examined from a particular host.

Many different species names have been applied to these forms. Three names have been applied to forms from pigs alone. A review of the taxonomic situation with respect to these *Endamoeba* has recently been given by Noble and Noble (1952), and will not be repeated here. These authors concluded that on the basis of morphological characters alone there is no justification for separating any of these forms into distinct species. However, since physiological characteristics of these amebae from different hosts have not been investigated, and since there are no data on cross infection experiments, they feel justified in combining the several forms into one species. They therefore "recognize existing specific names as indications of host identification rather than as evidence for morphological identity." On this basis, considering the three species named from pigs as identical, they accept the following names for parasites from the hosts indicated: *E. bovis* from cattle, *E. ovis* from sheep, and *E. polecki* from pigs and goats. Earlier in the paper they indicated their belief that *E. chattoni* from monkeys is identical with *E. polecki,* a view which supports that of Kessel and Johnstone (1949), who referred their parasites from monkeys and man to this species.

There is some question, as Noble and Noble stated, as to what name should be applied to the parasites from pigs, goats, monkeys, and man, for which they accepted the name *E. polecki,* and what name should be accepted if the forms from cattle and sheep are also included. The name *E. polecki* was first applied by Prowazek (1912) to amebae found in pigs, and in one case from a child in Saipan. Although, as Dobell (1919) first mentioned, Prowazek's description was very inadequate, and probably included a mixture of amebae from pigs and human beings confused with free-living species from stale human stools, Noble and Noble (1952) remarked

that Prowazek "undoubtedly described the common uninucleate cysts belonging to the genus *Entamoeba*." So, if all forms described from pigs constitute a single species, Prowazek's name is valid, and is not a *nomen nudum* as Hoare (1940) considered it. However, if the amebae with uninucleate cysts from ruminants as well as other hosts should be considered as constituting a single species, Noble and Noble's conclusion that the name *E. polecki* is applicable on the basis of priority is erroneous. *Amoeba bovis* Liebetanz, 1905 would have priority.

We agree with Noble and Noble that until more is known about the physiological character of some of these parasites, it is best to retain the species names as indications of host relationships. Since it has been generally accepted by recent workers (Kessel and Johnstone 1949; Lubinsky 1952; and Noble and Noble 1952) that the forms described from man and monkeys are identical with those in pigs, we believe that *Endamoeba polecki* Prowazek, 1912, is the correct name for the parasite here described.

The infection herein described is the only instance in which a single infection with an *Endamoeba* of *E. polecki* type has been observed at *frequent* intervals over a long period of time. The fact that the size of the cysts remained consistently large, i.e. 8.30 to 14.94 microns in stained smears, with an average of 11.62 microns, suggests the possibility that, as in the case of *E. histolytica* and, we believe, other amebae, there are races differing in the size of the cysts. The wide range in size reported by some workers (Nöller 1921 and 1922; Hoare 1940; and Noble and Noble 1952) suggests that they were lumping different races from different hosts, whereas the rather consistently small size reported by others (Cauchemez 1922; Smith 1910) indicate that they were dealing with a smaller race than the one here described. Nieschulz (1923, 1924) used size as a distinguishing characteristic between two species in pigs.

If Prowazek's (1912) report of this parasite from a child in Saipan constitutes a valid record, the present case is the fifth reported occurrence of *E. polecki* in man. Cases have been reported by Johnstone in California. Two of these were reported by Kessel and Johnstone (1949) and a third was mentioned by Dr. Johnstone in a personal communication. The one additional case was reported by Lubinsky (1952) in Pakistan. As Dr. Johnstone mentioned in his letter to the writer, this parasite may be more common in man than is suggested by known reports, and until the morphological characteristics of this species are better described the identification will be difficult and it will continue to be confused with *E. histolytica*.

SUMMARY

1. A case of *Endamoeba polecki* infection in an Egyptian was studied over a period of 33 months. The morphology of the trophozoite and cysts, living and stained, was studied in the stools of the subject and the trophozoites in culture, and compared with previous descriptions of this amoeba in both man and animals. The trophozoite stage of this species has not previously been described in man.

2. Attempts at culture in Difco's Bacto-Endamoeba medium were only temporarily successful.

3. Three amebacidal drugs—Emetine, Diodoquin, and Vioform—failed to have any effect on the course of the infection.

4. No evidence of pathological effect was observed during the course of the study.
5. This constitutes the fifth reported case of a human infection with this species.
6. The taxonomy and nomenclature of this parasite are discussed.

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Explanation of Plate

Fig. 1. Trophozoite of *E. polecki*. Note: bipolar thickening of chromatin on the nuclear membrane, vacuolated cytoplasm, ingested particles, small karyosome.

Figs. 3–6. Uninucleate cysts with inclusion mass and nuclei with large aggregated or solid karyosomes.

Fig. 2. Cyst containing large glycogen vacuole and numerous chromatoid bodies.

Fig. 3, 4. Cysts containing nuclei with stellate shape karyosomes.

Fig. 5. Cysts containing nuclei with solid disc type karyosome and inclusion mass with glycogen appearing area.

Fig. 6. Small cyst with no chromatoid material.

Figs. 7–9. Cysts showing absence of inclusion mass.

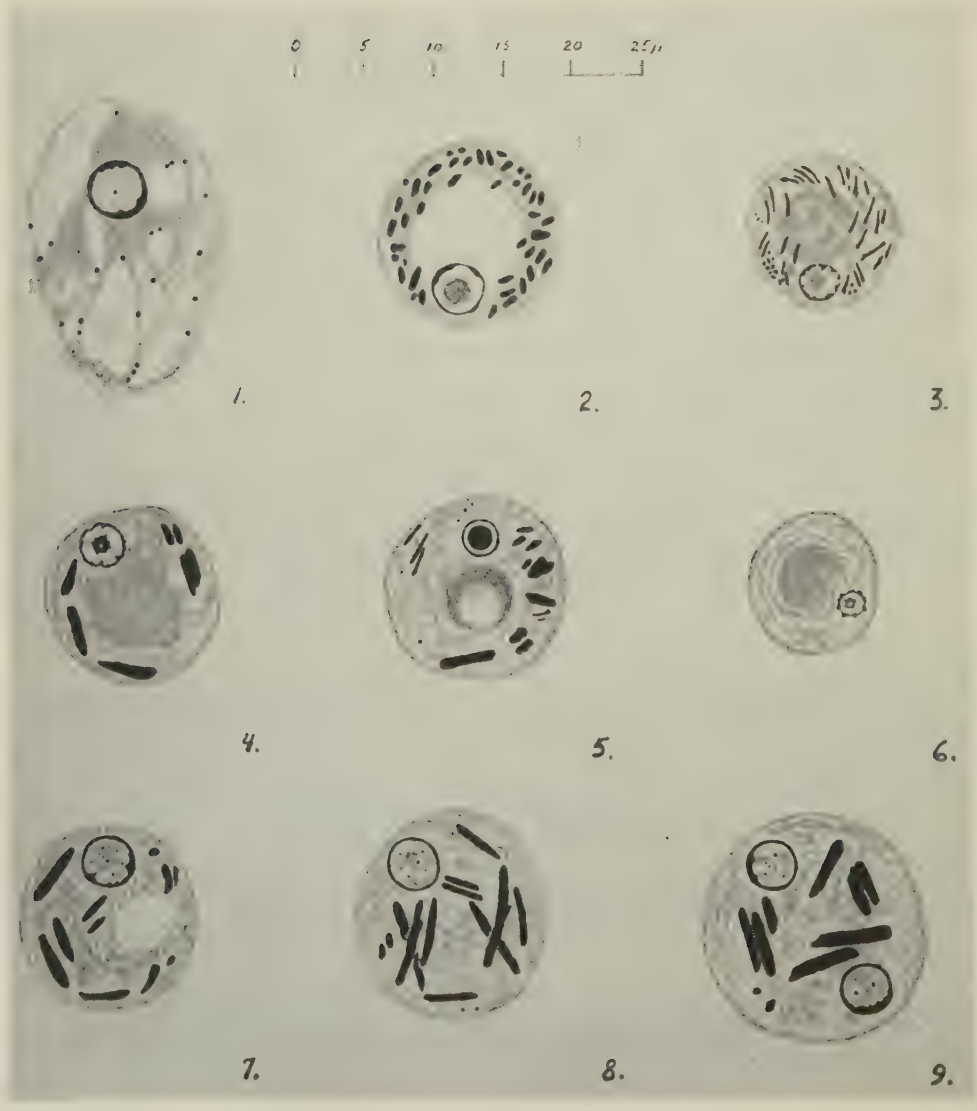
Figs. 7, 8. Uninucleate cyst containing nuclei with small central and slightly eccentric karyosomes.

Fig. 7. Cyst with small glycogen vacuole and irregular chromatin granules on the nuclear membrane.

Fig. 8. Cyst with evenly arranged beadlike chromatin formation on the nuclear membrane.

Fig. 9. Binucleate cyst.

PLATE I



BLOOD PARASITES OF BIRDS OF THE HIGH ROCKIES

REGINALD D. MANWELL*

As far as I am aware, there are no published reports of the blood parasites of birds of the High Rockies. Since environmental conditions are quite different in such regions, with very short summers, uniformly cold nights (often with heavy frost), and low daytime humidity combined with moderate warmth and much sunshine, with consequent differences in the species of blood-sucking arthropods (possible parasite vectors) encountered, it seemed worth while to examine all birds available, even though many of them migrate during the winter either to lower altitudes or more southern ranges. There is also a somewhat different avian fauna at high altitudes.

This study was done at the Rocky Mountain Biological Laboratory at Gothic (near Crested Butte), Colorado, at an altitude of 9,500 feet. Some of the birds were brought in from neighboring areas, up to 11,000 feet. The cliff swallows for the most part were taken from under the eaves of a small school building at Parkvale, a tiny hamlet a few miles west of Canon City, near the foot of Monarch Pass; some however were obtained under a cliff near Cement Creek, near Taylor Reservoir, some 25 miles from Gothic.

Birds were either trapped or shot, or—in the case of juveniles—removed from the nests. Blood smears, supplemented in some cases by organ smears, were stained with J.S.B. All blood smears were examined under oil for a minimum of 5 minutes. The organ smears, and in some cases the blood smears also, were often studied much longer.

Table 1 gives a summary of the species of birds included in the survey, and of the parasites found. The numbers are not large, but some of the results are of considerable interest and it seems worth while to report them, as the study cannot be continued in the near future.

The occurrence of an acute case of malaria in a Canada Jay (a non-migratory bird) was a surprise. The very low incidence of malaria in Western Robins (see footnote to Table 1) was also unexpected. Only one case was seen (*Plasmodium vaughani*). The incidence of malaria, especially *vaughani* malaria, is very high among these birds at lower altitudes. Since *vaughani* malaria does not show a clearly marked acute stage, it is impossible to know whether this was a recently acquired or a chronic infection. However the infrequency of this species of malaria among robins of this area may be due to the absence of the unknown insect vector.[1]

Inasmuch as exoerythrocytic stages have not yet been demonstrated for *Plas-*

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* From the Department of Zoology, Syracuse University, and the Rocky Mountain Biological Laboratory, Crested Butte, Colorado.

[1] Although attempts to transmit *Plasmodium vaughani* by the use of *Aedes aegypti* and *Culex pipiens* have so far been unsuccessful (Manwell, R. D., J. Parasit., **33**: 167, 1947), it is of considerable interest that a species of *Aedes* seems to be by far the most abundant mosquito in the Gothic area. In this connection, it may also be significant that *Plasmodium vaughani* occurs in the Japanese Hill Robin in Hawaii, where there are but 3 species of mosquitoes: *Aedes aegypti, A. albopictus,* and *Culex quinquefasciatus.* (Kartman, Leo, 1947—personal correspondence.) Thus it seems evident that the possible role as vectors of culicine mosquitoes of all these species should be reinvestigated.

modium vaughani, this robin was sacrificed, and organ smears carefully searched (lungs, brain, spleen, liver, and bone marrow), but with completely negative results.

Another fact of interest was the apparently complete absence of *Plasmodium polare* among the cliff swallows examined. This contrasts with the results obtained from cliff swallows of central New York, in which the incidence among the birds we have examined has been relatively high.

Of the 9 species included in the survey, blood parasites were most commonly encountered in the robins. Besides the single case of *vaughani* malaria, microfilariae, *Leucocytozoon, Haemoproteus,* and trypanosomes were found, the 3 last named in nestlings. This proves the incubation period to be not longer than 10

TABLE 1.—*Blood parasites of Rocky Mountain birds*

Bird		Number of Cases and Age**		Parasites**								Tot. Inf.***
Species	Common Name			Mf	T	L	H	Hg	Pc	Pv	Mx	
Selasphorus platycercus platycercus	Broad-tailed Humming Bird	6	Jv (0)	..	..	..	..	..	..	..	..	..
			Ad (6)	0	0	0	0	0	0	0	0	0
Perisoreus canadensis canadensis	Canada Jay	7	Jv (0)	..	..	..	..	..	..	..	..	..
			Ad (7)	0	0	3	1	0	1	0	1	4
Petrochelidon albifrons albifrons	Cliff Swallow	46	Jv (37)	0	0	0	0	2	0	0	0	2
			Ad (9)	0	0	0	1	6	0	0	1	6
Junco caniceps	Gray-headed Junco	2	Jv (0)	..	..	..	..	..	..	..	..	..
			Ad (2)	0	0	1	0	0	0	0	0	1
Melospiza lincolni lincolni	Lincoln's Sparrow	3	Jv (0)	..	..	..	..	..	..	..	..	..
			Ad (3)	0	1	0	1	0	0	0	1	1
Empedonax difficilis difficilis	Western Flycatcher	1	Jv (0)	..	..	..	..	..	..	..	..	..
			Ad (1)	0	0	0	0	0	0	0	0	0
**Turdus migratorius propinquus*	Western Robin	15	Jv (7)	0	1	5	2	0	0	0	2	5
			Ad (8)	1	1	5	3	0	0	1	4	6
Zonotrichia leucophrys leucophrys	White-crowned Sparrow	1	Jv (0)	..	..	..	..	..	..	..	..	..
			Ad (1)	1	1	1	0	0	0	0	1	1
Zonotrichia albicollis	White-throated Sparrow	1	Jv (0)	..	..	..	..	..	..	..	..	..
			Ad (1)	1	1	1	0	0	0	0	1	1
Totals		82		3	5	16	8	8	1	1	11	27

* The Eastern and Western Robin differ only in the outer tail feathers, which in the former are white. However an occasional robin seen in Gothic, including one of those caught, has a trace of white on these feathers, but all were regarded as of the latter subspecies.

** Abbreviations: Mf = microfilariae; T = trypanosomes; L = *Leucocytozoon;* H = Haemoproteus; Hg = haemogregarines; Pc = *Plasmodium cathemerium;* Pv = *P. vaughani;* Mx = mixed infections; Jv = juvenile; Ad = adult.

*** The numbers given are, in each case, the totals for the species listed, each mixed infection being counted as one.

days, and that the unknown vectors are present locally. It also suggests that these parasites may be commonly, and perhaps usually, acquired early in the lives of the hosts. Whether the infections result in a significant mortality is unknown, but they were quite severe in the nestlings observed.

The robins in which trypanosomiasis was found were sacrificed, in an attempt to find where the parasites were localized and where multiplication occurred. No dividing forms were seen in the blood, nor could any be found in the tissues. Neither was any special concentration observed in the bone marrow or lungs—organs in which they are often numerous in some species of birds. It is rather remarkable that no evidence of trypanosome multiplication could be found in the in-

fected nestling, for this was obviously a very recently acquired, and hence an acute case.

Reproducing stages of *Leucocytozoon* were seen only in the kidneys of infected robins, making them similar in this respect to *Leucocytozoon*-infected grackles.

No multiplicative stages of *Haemoproteus* were observed.

RESEARCH NOTES

A CASE OF ASPERGILLOSIS IN A SONG SPARROW

Birds are said by Conant *et al.* (Manual of Clinical Mycology, 1945) to be especially susceptible to infection with *Aspergillus,* and they remark that a series of penguins which were autopsied showed a 40 per cent incidence of aspergillosis. Our own experience, extending over a period of many years and including necropsy of a large number of birds of some 50 species, has shown little evidence that this is generally true in nature, or at least that cases are at all common in which the fungus is pathogenic enough to cause grossly visible lesions.

For this reason a case of aspergillosis in a song sparrow, which ended fatally, is of interest. The sparrow was caught in Fayetteville, N. Y., in a funnel trap, on July 24th, 1951, and kept in the laboratory for 10 days afterward in a cage with a number of other song sparrows. It died on August 3rd and autopsy showed one lung to be completely destroyed by a dense growth of *Aspergillus fumigatus.* The other lung appeared superficially normal. A blood smear showed profound anemia. Although the bird also showed a moderate infection of both *Haemoproteus* sp. and *Plasmodium cathemerium,* it is almost certain that the cause of death was aspergillosis.

This is the only case of aspergillosis (or pulmonary mycosis of any sort) we have ever seen in a song sparrow, or any other species of bird. Since its cage mates, which were also kept for varying lengths of time until they died in the laboratory, showed no evidence of mycosis it must be assumed that this song sparrow acquired its infection before capture.—Reginald D. Manwell, *Syracuse University, Syracuse, New York.*

SOME HELMINTHS OF WEST COAST URODELES

During the winters of 1952 and 1953 a study was made of the helminth fauna of some west coast urodeles, collected in Central California and the Willamette Valley of Oregon. The 99 animals examined included 4 *Aneides flavipunctatus,* 2 *Aneides ferreus,* 10 *Batrachoseps a. attenuatus,* 1 *Dicamptodon ensatus,* 33 *Ensatina e. escholtzii,* 3 *Ensatina escholtzii oreganus,* 34 *Triturus granulosus* and 12 *Triturus torosus.*

Five species of Nematoda and three species of Trematoda were recovered. New host records are marked with an asterisk (*). Distinction is made between the species of *Triturus* with regard to their habitats, i.e. aquatic or terrestrial. All other hosts, with the exception of *Dicamptodon,* belong to the family Plethodontidae and are restricted, in these areas, to terrestrial habitats; the one adult specimen of *Dicamptodon ensatus* was captured in a terrestrial situation.

TREMATODA: Brachycoelium salamandrae (Frölich 1789) from intestines of 1 of 6 terrestrial *T. granulosus**, Marion Co., Ore., and one of three *Ensatina escholtzii oreganus**, Benton Co., Ore.

Cephalouterina dicamptodoni Senger and Macy 1953 from intestine of 1 *Dicamptodon ensatus,* Benton Co., Ore.

Megalodiscus americanus Chandler 1923, from rectum of 6 of 28 aquatic *T. granulosus**, Benton Co., Ore.

NEMATODA: Cosmocercoides dukae (Holl 1928) from intestine and rectum of all of 12 aquatic *T. torosus,* Contra Costa Co., Calif., 26 of 28 aquatic and all 6 terrestrial *T. granulosus**, Benton Co., Ore., and all of 6 terrestrial *T. granulosus**, Marion Co., Ore.

Hedruris siredonis Baird 1858 from the stomach of 8 of 28 aquatic *T. granulosus** and 3 of 6 terrestrial animals of the same species, Benton Co., Ore.

Oxyuris dubia Leidy 1856, rectum of all 33 *Ensatina e. escholtzii**, Sonoma Co., Calif., 1 of 3 *Ensatina e. escholtzii oreganus**, Benton Co., Ore., 3 of 4 *Aneides ferreus**, Benton Co., Ore., and 3 of 10 *Batrachoseps a. attenuatus**, San Francisco Co., Calif.

Oxyuris magnivulvaris Rankin 1937 from rectum of 1 of 2 *Aneides flavipunctatus**, Marin Co., Calif.

Rhabdias sp. from lungs of 2 of 12 aquatic *T. torosus,* Contra Costa Co., Calif.—Donald L. Lehmann, *Department of Zoology, Oregon State College.*

IXODES (*CERATIXODES*) *URIAE* WHITE, 1852, PARASITIZING PENGUINS AND SEA BIRDS IN THE FALKLAND ISLANDS (IXODOIDEA, IXODIDAE)

The following records, representing a comparatively sizable collection of *Ixodes* (*Ceratixodes*) *uriae* White, 1852, are of interest in that they indicate the variety of hosts infected by this tick in a single locality, the numbers, stage, and sex of ticks on any one host, and parts of the host in which these parasites shelter.

All specimens were collected on Kidney Island, Falkland Islands, by the British Grahamland Expedition, 1934–1936. No other species of ticks were taken. The collection was sent to me for identification by British Museum (Natural History). I am indebted to the Director and to Mr. E. Browning and Dr. G. Owen Evans of that Institution for the privilege of examining this material and making this report. Mr. Browning kindly checked host and locality records.

The Falkland Islands are in the South Atlantic Ocean about 400 miles off the southern tip of South America.

From Magellanic Penguin, *Spheniscus magellanicus* (Forster)

No. 438 12 nymphs from ear cavities and hood patch, 3 December, 1936.

No. 464 1 larva, 9 nymphs, 4 December, 1936.

No. 275 13 nymphs, 30 November, 1934.

From Rockhopper Penguin, *Eudyptes crestatus* (J. F. Miller)

No. 18 1 nymph, 2 ♀♀, 8 December, 1934.

No. 415 2 larvae, 42 nymphs, from ear cavities and hood patch, 30 November, 1936.

No. 454 15 nymphs, 1 ♀, from ear cavities and hood patch, 3 December, 1936.

From Dolphin Gull, *Larus scoresbyi* Traill.

No. 13 2 ♀♀, 8 December, 1934.

From King Shag or Cormorant, *Phalacrocorax albiventer* (Lesson)

No. 15a 1 larva, 2 nymphs, inside mouth, 8 December, 1934.

From Rock Shag or Cormorant, *Phalacrocorax magellanicus* (Gmelin)

No. 16 1 nymph, 8 December, 1934.

British Museum (Natural History) accession numbers for this collection are: 1953.10.7.1–61.

The synonymy and some biological features of this species have recently been reviewed by Zumpt (1952, Austral. Nat. Ant. Res. Exped., Rep., Ser. B, **1**: 12–20), who stresses the need for additional material from different places and hosts for comparative morphological studies.

The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.—H. Hoogstraal, *Head, Department of Medical Zoology, U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.*

THE OCCURRENCE OF *PLASMODIUM RELICTUM* IN THE WOOD DUCK (*AIX SPONSA*)

In the summer of 1951 the investigator, in cooperation with the Pennsylvania State Game Commission, began a survey of Pennsylvania birds for blood parasites.

In the fall of 1951 a hunter contributed a freshly killed wood duck—*Aix sponsa*. The blood of the wood duck contained *Plasmodium relictum* Grassi and Feletti, *Haemoproteus nettionis* and an unidentified microfilaria. The parasites were identified by Dr. Carlton M. Herman, Wildlife Pathologist of the Patuxent Research Refuge at Laurel, Maryland.

The occurrence of *Plasmodium relictum* in the wood duck has not been reported in the literature indicating the possibility of a new host record.—J. Eugene Mielcarek, *Kansas City College of Osteopathy and Surgery, Kansas City, Missouri.*

THE OCCURRENCE OF *ORNITHODIPLOSTOMUM PTYCHOCHEILUS* (FAUST) (TREMATODA: STRIGEIDA) IN FISH AND BIRDS

The metacercaria of *Ornithodiplostomum ptychocheilus* has been reported by Faust (1918; Illinois Biol. Monogr. **4** (1): 1–120) from mesenteries of the squawfish, *Ptychocheilus oregonensis* (Richardson), and by Hughes and Piszcek (1928; Jour. Parasit. **15**: 58–62) and Van Haitsma (1930; Tr. Amer. Micr. Soc. **49**: 140–153) in the northeastern sand shiner, *Notropis deliciosus stramineus* (Cope). Van Haitsma (1930) found the adults in mergansers, *Mergus americanus, M. serrator, Lophodytes cucullatus,* and the old squaw duck, *Harelda hyemalis.* He also reared the metacercaria to the adult in young domestic ducks. Lyster (1940; Can. J. Research **18D**: 79–82) reported this worm as a cyathocotylid, *Paracoenogonimus katsuradi* n. sp., from *L. cucullatus* in Canada, but Dubois (1946; Ext. Actes Soc. Helvet. Sci. Nat. Zurich: 153–154) placed this name in synonymy with *O. ptychocheilus.*

During an examination of fish from Turtle River, Arvilla, North Dakota, the writer found the metacercariae of *O. ptychocheilus* sparingly (0–52, usually 1 or 2) in the mesenteries of four new hosts: the northern common shiner, *Notropis cornutus frontalis* (Agassiz), 6 of 7 infected; the central bigmouth shiner, *Notropis d. dorsalis* (Agassiz), 7 of 17; the northern fathead minnow, *Pimephales p. promelas* (Raf.), 1 of 3; and the northern creek chub, *Semotilus a. atromaculatus* (Mitchell), 17 of 19. All except *N. d. dorsalis* were infected with *Posthodiplostomum m. minimum* in the same location, i.e., encysted in the mesenteries. However, *P. minimum* was not found in a total of 41 *N. d. dorsalis* examined. The cysts of *O. ptychocheilus* were also found in the extra-dural space of the cranial cavity of 7 of 12 *N. c. frontalis,* but none were found in that location in any of the other 8 spp. of fish examined from Turtle River. They were found, however, in the cranial cavity of *P.p. promelas* from the English Coulee and Kelly's Slough, Grand Forks, North Dakota. The metacercariae from this unusual location appeared to be identical with the others. The number of larvae present ranged from 0 to 10. Dr. L. O. Nolf and I have also observed this metacercaria in cyprinids at Woodruff, Wisconsin, in 1948.

To verify the species, the metacercariae of *O. ptychocheilus* were reared to egg-producing adults in unfed chicks in 2 days, following the method of Ferguson (1938; Jour. Parasitol. **24** (6) Suppl.: 31). The adults differed from the description of Van Haitsma (1930) only in the variation of the "strigeid constriction." He stated that the hindbody is not distinctly set off by a groove from the forebody and did not report his fixing methods or state if the worms were observed alive. In my material the constriction is evident but not pronounced in the living worms; there is a suggestion of it after hot A.F.A. fixation but it is lost following cold fixation and freezing. Because of the confusion this characteristic causes under different conditions, the description should be amended slightly to read "strigeid constriction reduced but evident when alive and following hot fixation."

I wish to thank Professor G. R. LaRue, Zoological Division, Bureau of Animal Industry, Beltsville, Maryland, for reading the manuscript, and Dr. W. F. Potter of the University of North Dakota Medical School for making certain facilities available for this study.—GLENN L. HOFFMAN, *University of North Dakota.*

PRURITUS PRODUCED BY THE ZOOEA LARVAE OF ONE OF THE PORCELAIN CRABS

During the first and second weeks of September of last year, the author's attention was called to a curious dermal affliction of children swimming at the entrance to Quisset Harbor, a small embayment emptying into Buzzards Bay on Cape Cod, Massachusetts. Many of the youngsters under ten years of age bathing at this beach complained of stinging and itching both in the water and for a short while after they had dried themselves and dressed.

It was thought at first that a cercarial dermatitis had been contracted similar to that caused by *Microbilharzia variglandis* (Miller and Northup, 1926) described by Stunkard and Hinchliffe in 1952 (J. Parasit. **38**: 248–265) for neighboring Narragansett Bay. Examination of the irritated areas of the skin revealed no lesions, macules or papules, nor was there any zone of erythema visible. In addition, all irritation disappeared in every case about an hour after the affected youngsters had dressed. The lack of an eruption or visible edema fairly well eliminated the suspicion that the irritation was caused by cercariae; and in addition, a previous twelve month survey of nearby Gansett Cove by Miller and Northup (1926, Biol. Bull. **50**: 490–508) revealed only three of 8,875 snails (*Nassa obsoleta*) possessing *M. variglandis.*

A second possibility, that the swimmers had been affected by the nematocysts of jelly-fish, was also investigated. Again the absence of both visible and prolonged irritation tended to remove these coelenterates as the cause of the difficulty. Moreover, plankton tows and general observation revealed three small species of hydromedusae and the ctenophore, *Mnemiopsis leidy,* as the only jelly-fish present at that time.

Careful examination of the irritated areas of the complainers showed minute, white, somewhat translucent, hairlike projections at the places where the slight stinging was felt. When these small, feathery barbs were removed and examined it was found that they were all the last stage of the zooea larva of one of the little porcelain crabs, *Polyonyx macrocheles.* In this larva, the carapace has two long spines behind, and a rostral spine in front, slightly barbed along its entire length. The rostral spine is several times as long as the body of the animal. The rostral spines of the porcellanid larvae had been embedded in the skins of the swimmers. Collections with a tow net revealed an unusually large swarm of these larvae in the plankton at the time of the attacks.

It is thought that this is the first report of porcellanid larvae interfering with human swimmers. In this instance the children alone felt the effects of rostral penetration, presumably because of their less cornified and thinner epidermis. Penetration through the outer epithelium produced the sensation of light pinpricks similar to that of a small splinter or thorn, on the

shoulders, arms, and thighs. Slight rubbing of the irritated areas after the skin had dried removed the entire animal.—DONALD J. ZINN, *University of Rhode Island, Kingston, Rhode Island.*

A USEFUL TECHNIQUE FOR EXPOSURE OF ANIMALS TO CERCARIAE OF *SCHISTOSOMA JAPONICUM*

Various techniques for exposure of laboratory animals to *Schistosoma japonicum* have been described (Rose and Koh, 1936, Tung-Chi Med. Monatsschr. Kongr.-Heft. pp. 78–102; Kato, 1939, Kitasato Arch. Exp. Med. **16:** 340–342; Pan, Kaufman and Hunter, 1951, J. Lab. and Clin. Med. **37:** 817–819; and others); each has certain advantages as well as disadvantages depending on the experimental design. A method used by us, incorporating certain features of previously developed techniques, has been found to give better animal infections and better protection for the operator. The method consists essentially of applying cercariae by means of a fine hair loop to the skin of anesthetized animals. Description of the technique as here given applies to the exposure of mice; minor changes will adapt it for use on other animals.

The hair loop is made from a human hair 5 to 6 cm. in length by folding it so that the ends are brought together; then while the ends are held between the thumb and forefinger the hair is twisted by rolling action so that a loop is formed at the folded end. The size of the loop thus formed may be controlled by varying the amount of twisting. Duco cement is then spread along the twisted portion of the hair with the point of a dissecting needle. When the cement is hardened the shank of the loop is trimmed to 5 mm. and attached to the end of an applicator stick with a drop of cement. It is desirable to prepare 4 or 5 loops ranging in size from 2 to 5 mm. in diameter.

Prior to exposure, mice are prepared by closely clipping the hair over the abdominal surface. The mice are then weighed to the nearest gram and the required dosage of sodium nembutal computed. A simple dosage schedule, devised by Dr. George Luttermoser of this Laboratory, gives adequate anesthesia and when carefully followed death due to anesthesia is rare. A working solution, prepared by adding 1.5 ml. of veterinary sodium nembutal (60 mg. per cc.) to 9 ml. of sterile normal saline, is injected intraperitoneally at the rate of .01 ml. per gram body weight with a 1 ml. tuberculin syringe and a 1¼ in.-23 gauge needle. When given at this rate anesthesia is obtained for 1 to 2 hours. This dosage schedule may also be used for hamsters.

After the mice are anesthetized and placed in a supine position, their abdomens are rubbed with moistened fingers to wet the skin. Care should be taken to apply only enough water to moisten the skin as an excess makes discharging of the cercaria from the loop difficult. Under a dissecting microscope the cercariae are looped off the surface of the water. Then by focusing the microscope on the loop the cercariae may be counted before they are applied to the skin of a mouse. After the desired number of cercariae have been transferred, several drops of water are added to keep the skin wet while the cercariae are penetrating. Mice may then be returned to their cages in a supine position. As anesthesia lasts for more than an hour sufficient time is afforded for adequate penetration before the mice regain consciousness.

Uniformity of infection and the number of worms coming to maturity compare favorably with results by other methods. From 64 mice exposed to 50 cercariae each, an average of 64.3 per cent, ranging from 41 to 94 per cent, of the cercariae to which each mouse was exposed was recovered as adult worms. This represents a higher percentage return than reported for other methods of exposure.—WILLIAM B. DEWITT, *U. S. Department of Health, Education, and Welfare, National Institutes of Health, Bethesda, Maryland.*

A NEW LOCALITY RECORD IN AFRICA FOR THE TICK PARASITE, *HUNTERELLUS HOOKERI* HOWARD

In September 1953, the author had occasion to do some field work in the vicinity of Mombasa, on the Kenya Coast of the Indian Ocean, in company with Dr. S. F. Barnett, Acting Director of the Veterinary Research Laboratory, Kabete, Kenya. These studies were made while the writer was a consultant on "Kenya tick typhus" to U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, Captain A. R. Higgins, Director. Ticks and canine blood samples were taken with the assistance of the local veterinary staff in Mombasa and further samples were forwarded by Dr. William Pratt of that station.

Each of five engorged nymphs of *Rhipicephalus sanguineus* collected by Dr. Pratt from a dog at Bambur, four miles north of Mombasa, and carried to Montana in humidified tubes, proved to be infested with the minute, wasplike parasite, *Hunterellus hookeri* Howard. Eight, seven, and seven adult, fully winged parasites emerged from three of these about a month after collection. The parasites in the two remaining nymphs failed to emerge, perhaps because of vicissitudes of prolonged travel, but four winged specimens were dissected out of the shells of each of these two nymphal ticks.

This locality is remote from those previously recorded in Africa for this tick parasite species (several places in South Africa, Cooley and Kohls, 1934, Fifth Pacific Science Congress, **5**: 3375; and Nigeria, West Africa, Philip, 1931, Public Health Reports, **46**: 2168). In addition, the parasite has been reported from England, France, Eastern Siberia, India, Indo-China, Brazil, Cuba, Mexico, and from widely scattered points in the United States, such spread perhaps aided in times past by movement of tick-infested dogs. The adult parasites are weak fliers, and are more at home running about in the fur and hair of suitable vertebrate hosts.

In spite of the wide climatic adaptability of this parasite previously pointed out by the writer, the practicality of using it as a means of more than incidental biological control of ticks in any locality has yet to be demonstrated. Many species of ticks have been found naturally parasitized; however, dogs in the vicinity of Mombasa, as in other areas where the parasite abounds, continue to be heavily tick infested.

This additional locality increases considerably the known natural range of *H. hookeri* in Africa. Dr. O. G. H. Fiedler confirmed the identity of these parasites (6 females and 2 males in one lot) and writes of its discovery in the same host in Uganda. He has described a new species, *H. theilerae,* from ticks in South Africa which is quite distinct from *H. hookeri.* Identification of the nymphal ticks as *R. sanguineus* was confirmed by Harry Hoogstraal of NAMRU 3, Cairo, and Dr. Gertrude Theiler, Onderstepoort, South Africa.—Cornelius B. Philip, *Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Rocky Mountain Laboratory, Hamilton, Montana.*

EXPERIMENTAL STUDIES ON THE TRANSMISSION AND PATHOGENICITY OF *DERMOCYSTIDIUM MARINUM,* A FUNGOUS PARASITE OF OYSTERS

Dermocystidium marinum Mackin, Owen and Collier, has been transmitted to uninfected *Crassostrea virginica* (Gmelin) by two methods. One method, designated as "proximity" method, consisted of placing the uninfected oysters in aquaria with living, infected oysters; and the other, designated as "feeding" method, consisted of placing finely minced infected oyster tissues into the water of aquaria containing uninfected oysters. The studies were carried out in closed aquaria containing ten liters of sea water which was aerated continuously and renewed at weekly intervals. The experiments, which lasted nearly 5 months, were started on December 9, 1952, and discontinued on May 2, 1953. The uninfected oysters were obtained from Connecticut, the infected ones from Louisiana and Texas. All of the dead and surviving oysters were diagnosed for *D. marinum* by means of the thioglycollate culture technique (Ray, Science **116**: 360–361, 1952) and the intensity of infection was estimated.

The "proximity" method resulted in the infection of 38 (95 per cent) and the death of 20 (50%) of 40 Milford oysters in the experimental aquaria. Twelve (60%) of the dead oysters and 15 (75%) of the survivors were acutely infected (intensity of infection moderate or greater). On the contrary, the 15 Milford oysters in the control aquarium remained free of the parasite and only 3 (20%) of them died.

The incidence of infection obtained by the "feeding" method was similar to that of the "proximity" method. Twenty-six (87%) of 30 Milford oysters became infected and 25 (83%) of them died, whereas the 15 Milford oysters in the control aquarium were negative for *D. marinum* and only 6 (40%) of them died. The experimental oysters received 5 "feedings" of minces of variously infected oysters at irregular intervals, while the controls received only 1 "feeding" of uninfected tissue mince. The "proximity" method required 16 weeks to produce the first acutely infected dead oyster, while only 9 weeks were required with the "feeding" method.

The results show that *D. marinum* may be effectively transmitted by both methods described and they suggest that *D. marinum* may be transmitted directly. The increased mortality rate of the experimentally infected oysters (2.5 times greater in the "proximity" study and 2 times greater in the "feeding" study) over that of the uninfected control oysters indicates that this parasite is pathogenic for oysters under the conditions of the experiments.—Sammy M. Ray, *Biology Department, The Rice Institute.*